A STUDY OF ANAEMIA

AND

CHANGES IN THE BLOOD VOLUME

DURING NEPHRITIS IN CHILDREN.

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By

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PREFACE.

The problem presented by the peculiarly intractable form of anaemia associated with nephritis has been a subject of comment and investigation by many workers in adult medicine but there has been little detailed study of the blood changes in the nephritis of childhood although at this age period complicating factors are less common than in adults.

The present study was suggested to me by Professor G.B. Fleming and was undertaken in an attempt to gain new information on some of the features of this type of anaemia.

The work has been done in the wards, biochemical laboratory, and pathology department of the Royal Hospital for Sick Children, Glasgow, during the tenure of a McCunn Research Scholarship and later of a Hall Tutorial Fellowship and part of it has already been published in the Archives of Disease in Childhood, 1942, 17, 1, under the title of "Anaemia in Nephritis" and a further part is in the press for the Archives of Disease in Childhood, 1947, under the title of "The Blood Volume in Nephritis".

It is a pleasure to express my indebtedness to Professor G.B. Fleming for his constant advice and encouragement and for much helpful criticism throughout the course of the study. I am also indebted to Dr. G. Montgomery for assistance in the experiments on rabbits and for/

for post mortem reports, and to Professor Noah Morris and Miss O.D. Peden for advice on the biochemical methods.

I have also to thank Dr. Stanley Graham for permission to study cases in his wards.

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INTRODUCTION.

The accuracy and comprehensiveness of Richard Bright's original description of kidney disease is so outstanding that almost every study of nephritis or of related processes, begins by acknowledging the opinions that he expressed in his celebrated reports (Bright, 1836). Even the original recognition of anaemia as a feature of nephritis has been attributed to Bright in view of his statement that 'after a time, the healthy colour of the countenance fades' and his inclusion in the case history of most of his patients of observations such as 'of a pallid countenance'; 'with a pale complexion'. His recognition that the patients were anaemic is further confirmed by his advice that bleeding should usually be avoided because of 'the peculiar pallid hue'.

In 1841, Christison studied anaemia in chronic nephritis by a method of gravity sedimentation of defibrinated blood and the results that he obtained correspond to red cell counts of 3 to 3.5 millions per c.mm. In 1878, Leichtenstern, working in Germany, reported a considerable anaemia in patients with chronic nephritis. These were early observations before the different stages and types of renal lesion had been defined and before the modern methods of blood analysis had come into common use. In spite of the early recognition of the association between anaemia/

anaemia and renal disease it has not yet been clearly shown in which type of the disease the anaemia is most in evidence nor is there any uniformity of opinion regarding the nature of the anaemia when it does occur. The present investigation was undertaken in an attempt to gain fresh information on these subjects and on related problems which arose during the course of study.

It was recognised that the first essential was to select a suitable classification of nephritis which would permit separation of the cases into reasonably distinct clinical groups. The classification that has been adopted is based largely on those of Volhard and Fahr (1914) and Boyd (1944) and the disease is divided into four groups. Acute Haemorrhagic Nephritis, Chronic Haemorrhagic Nephritis, Nephrotic Nephritis, and Nephrosclerosis. In order to avoid frequent repetition, a brief description of each of these clinical syndromes is given at this point.

Acute haemorrhagic nephritis is a disease characterised by a sudden onset, often two to three weeks after a streptococcal infection, with constitutional symptoms such as fever, headache, vomiting, anorexia and sometimes pain in the loins. There is a slight or moderate degree of oedema at the beginning of the illness and at the same time the blood pressure is moderately raised. There is a mild azotaemia early in the disease and the urine is diminished in volume and contains albumin, blood, and hyaline,/

hyaline, granular, and blood casts. Recovery is usually rapid and all but one case in this group had recovered within three months. The single exception did not recover from the albuminuria for seven months.

Chronic haemorrhagic nephritis is the sequel to an acute haemorrhagic nephritis which has not proceeded to recovery. In these patients there is a clear history of an initial attack of acute nephritis following which the child was never perfectly well. The albuminuria continues and exacerbations occur from time to time. There is a more or less continuous moderate hyperpiesia ranging from 120 mm. Hg. to 140 mm. Hg., a moderate but increasing azotaemia, and oedema which varies in amount from time to time and is often absent. The urine contains albumin, red cells and casts.

Nephrosclerosis differs from these diseases in that the onset is insidious, there is neither haematuria nor oedema and the systolic blood pressure is much higher. Attacks of headache, vomiting and convulsions occur and there are retinal haemorrhages and ultimately papilloedema. The urine contains a trace of albumin but usually no red cells or casts and is often increased in volume. There is moderate or severe azotaemia.

The cases of renal dwarfism are included in this group though they have well known characteristics peculiar to themselves and differ in a number of ways from the ordinary/

ordinary type of nephrosclerosis. With the exception of the renal dwarfs, all the patients in this group had at one time or another a systolic blood pressure over 200 mm.Hg. and all had well marked neuro-retinitis and retinal haemorrhages.

The introduction of nephrotic nephritis as a separate group in this series is considered quite essential to the interpretation of the results to be described. However, it is recognised that many authorities doubt the specific nature of this disease and consider that it is an oedematous stage of subacute or chronic haemorrhagic nephritis. The disease is insidious in onset with massive oedema and severe albuminuria as prominent features. There is no haematuria, no azotaemia, and the blood pressure is normal. The serum proteins are very low (with reversal of the albumin-globulin ratio) and the blood cholesterol is often much increased. While oedema is increasing there is malaise, backache and oliguria. There is a striking susceptibility to acute pyogenic infections.

The routine adopted in the investigation of each case for the purposes of this study followed the same pattern though the frequency of examination and certain other details were modified at times to suit special purposes.

In the first place, a general examination of all the/

the systems was carried out and the diagnosis and classification of nephritis established on the usual lines. The special examinations relating to the blood were commenced as soon as possible after the patient was admitted to Hospital and consisted of the following routine. A red cell count, leucocyte count and haemoglobin estimation was made on capillary blood; a reticulocyte count and a differential white cell count were made; the blood pressure was taken, the weight noted and the degree of oedema recorded by empirical signs (Rennie, 1933). A 10 c.c. sample of blood was withdrawn from one of the elbow veins with as little venous engorgement as possible; 7 or 8 c.c. of the blood were added to 2 c.c. of 1.1% isotonic neutral potassium oxalate, 2 c.c. added to a little powdered oxalate, and the remainder allowed to clot. On the sample of blood added to powdered oxalate, red cell and white cell counts and haemoglobin estimations were made for comparison with the results obtained at the same time with capillary blood. Using the remainder of the blood, estimation of the non-protein nitrogen, blood and plasma chlorides, haematocrit red cell volume and serum protein, and the Van den Bergh reaction were carried out.

A twenty-four hour collection of urine was made commencing on the day of the blood examination and ending on the following day. The quantity and specific gravity of the urine was measured and a quantitative estimation of the albumin,/

albumin, blood, and chloride present was made, and a specimen was tested for the presence of excessive urobilin. In doing blood counts, certain details of technique were carried out in order to ensure the greatest degree of accuracy. The parts of the body usually used for obtaining blood are the lobe of the ear, the pulp of the finger, and in infants the heel, and the selected part may be gently cleaned with methylated spirit avoiding any brisk rubbing or squeezing. A Hagendorn needle is plunged sharply into the tissues and withdrawn. The first drop of blood is wiped off and samples taken from subsequent drops. All these precautions were observed in the present investigation and the lobe of the ear was used in each case. For each count, two red cell pipettes were filled, one white cell pipette, and the haemoglobinometer pipette. Leitz standard haemocytometer pipettes were used and the counts were done on a Zeiss counting chamber with improved Neubauer ruling. The haemoglobinometer was of the Haldane type and was manufactured by Messrs. Hawksley & Co., of London, who checked the accuracy of the colour before the commencement of the experiments. 100% on the instrument was equivalent to 13.8 grams of Haemoglobin %. In making the counts the red cell pipettes were shaken by hand so as to mix their contents thoroughly and about a third of the contents of each was allowed to flow out by gravity before putting/

putting a drop under the coverslip for counting. The average of the count from each pipette was taken as the correct result but if the two counts did not agree within 200,000 red blood cells per c.mm. they were rejected and the entire process repeated. A single white cell pipette was filled and a duplicate count done from it. Haemoglobin was estimated by Haldane's method in which oxyhaemoglobin is converted to carboxyhaemoglobin by coal gas. Reticulocytes were counted in films stained with brilliant cresyl blue and the differential white cell counts were made on films stained with Leishman's stain.

The haematocrit packed cell volume was determined by adding 7 or 8 c.c. of blood to 2 c.c. of 1.1% neutral potassium oxalate (Graff & Clark,1931) in a 15 c.c. graduated centrifuge tube and centrifugalisation at 2,500 revolutions per minute for thirty minutes. The experimental evidence upon which most of these technical refinements are based has been provided by the work of Wintrobe (1934).

The non-protein nitrogen estimations were done on 0.2 c.c. of blood by a micro-Keldhal method (Folin & Svedberg,1930). The proteins were removed by sulphate tungstate and sulphuric acid, phosphoric acid, and copper sulphate. The non-protein nitrogen was estimated colorimetrically after Nesslerisation against a known standard solution of ammonium sulphate.

The chloride content of 1 c.c. of blood or plasma was estimated by Whitehorns modification of the Vollhard titration method (van Slyke, 1924). 1 c.c. of silver nitrate was added to precipitate the chloride and 10 c.c. of nitric acid to digest the fibrin. After 4 hours on the hot plate, the chloride content of the fluid was calculated by titration with potassium sulphocyanide.

The indirect Van den Bergh test was used (Van den Bergh, 1924). The total serum protein was estimated by the dipping refractometer.

The amount of albumin in the urine was estimated by the standard Esbach method.

The urobilin and urobilinogen content of the urine was estimated by Schlesinger's fluorescence test (Harrison, 1930) and by Wallace & Diamond's (1925) modification of Ehrlich's aldehyde reaction.

The degree of haematuria was estimated by doing duplicate red cell counts on carefully mixed 24 hour specimens of urine. The number of cells in one cubic millimetre of urine was counted and the result multiplied by the volume of urine in cubic millimetres (see Part 4 of this thesis).

In addition to these routine tests a number of other methods of study were used for particular purposes and these are described in the sections to which they apply.

PART I.

THE EFFECT OF OEDEMA ON THE ACCURACY OF RED CELL COUNTS DONE ON CAPILLARY BLOOD.

By following a careful procedure in blood examination, an endeavour has been made to eliminate error of technique as far as possible but there remains a potential source of error dependent on the waterlogged state of some of the patients' tissues which, it was thought, might lead to fallacious results. When withdrawing blood by skin puncture in oedematous patients, it is possible that there may be an artificial dilution of the blood by admixture with fluid from the oedematous tissues. In order to determine whether this in fact did occur, red cell counts were done simultaneously on blood obtained from a needle puncture of the lobe of the ear and on blood withdrawn from an arm vein. The venous sample was obtained with as little stasis as possible, though in some very oedematous children a considerable amount of venous engorgement was required in order to find a vein and put the needle into it. The blood having been withdrawn in a syringe, about 2 c.c. was transferred to a small glass tube containing a little powdered oxalate and then carefully but thoroughly mixed and used immediately for cell counts.

Eighty-three/

Eighty-three of these duplicate counts, capillary and venous, were done on thirteen subjects. Thirty-nine of the counts were done at a time when the patients had no oedema as shown by the absence of putting on pressure, or puffiness of the face, and by a stationery weight. Forty-four were done during a phase of oedema which varied in degree from simple putting on pressure over the shins to gross anasarca. The results obtained (Tables 1 and 2) show that in the non-oedematous group the capillary blood is 159,000 (±25,000) red blood cells per c.mm. more concentrated than the venous blood. If excessive tissue fluid produces dilution of capillary blood during its collection, then the increased red cell concentration of the capillary blood in the oedematous group should be less than 159,000 per c.mm. The figures in Table 1 reveal that the mean difference in the oedematous group is indeed less than 159,000, namely, 59,000 (± 38,000) red blood cells per c.mm. That is to say, the presence of oedema has, on the average, produced a false dilution of 100,000 red blood cells per c.mm. and it remains to calculate whether such a figure is statistically significant. To be significant, the diminution in concentration of capillary blood should be more than twice the standard error of the difference between the mean differences which is 46,000. This proved to be the case in the present instance.

This/

TABLE 1.

OEDEMATOUS GROUP.		NON-OEDEMATOUS GROUP.	
Capillary Blood.	Venous Blood.	Capillary Blood.	Venous Blood.
3.810	3.640	2.460	2.595
3.625	3.580	4.760	4.740
3.530	3.550	4.400	4.225
3.210	3.250	4.425	4.180
2.980	2.920	4.525	4.185
3.385	3.245	2.970	2.875
3.270	3.250	3.315	3.350
3.530	3.810	4.025	4.000
3.824	3.155	5.020	4.965
3.000	2.855	4.650	4.325
3.180	3.165	4.665	4.630
5.495	5.205	4.700	4.275
4.910	4.775	4.410	4.140
5.120	5.425	4.425	4.505
4.750	5.225	3.990	3.820
4.690	4.855	3.740	3.500
4.755	4.320	4.060	4.080
5.135	4.820	4.210	3.585
5.005	5.090	4.065	3.915
5.180	4.930	3.520	3.540
5.135	5.335	3.965	3.425
4.820	5.010	3.825	3.770
6.185	5.860	3.865	3.400
4.715	4.925	3.630	3.500
4.090	4.275	3.700	3.875
4.085	3.995	3.730	3.810
4.110	4.160	3.760	3.510
4.685	4.330	3.875	4.010
3.380	3.150	3.710	3.670
3.735	4.135	4.107	4.100
4.785	4.875	4.010	3.580
4.650	4.190	3.810	3.720
3.665	3.530	3.730	3.600
3.680	3.610	4.330	4.385
3.940	3.650	3.955	4.030
3.445	3.280	3.800	3.525
3.680	3.400	3.655	3.630
4.010	3.720	3.965	3.810
4.055	4.340	3.875	3.685
4.775	5.255		
3.585	3.735		
3.440	3.490		
4.130	3.485		
4.480	3.880		
Averages		Averages	
4.164	4.105	3.991	3.832

mean difference 59,000 (±38,000) (S.D. 254,000)

mean difference 159,000 (± 25,000) (S.D. 155,000)

The individual cell counts done on capillary and venous blood in oedematous and non-oedematous individuals.

Figures are millions of R.B.C. per c.ml.

1. The standard error of the difference between the mean differences = 46,000.

2. The increased concentration in the capillary blood in the oedematous group is 100,000 less than in the non-oedematous group and is therefore statistically significant.

TABLE 2.

AVERAGES OBTAINED OF EIGHTY-THREE DUPLICATED BLOOD COUNTS ON VENOUS AND CAPILLARY BLOOD IN OEDEMATOUS AND IN NON-OEDEMATOUS INDIVIDUALS.

OEDEMATOUS GROUP (44 COUNTS)		NON-OEDEMATOUS GROUP (39 COUNTS)	
Capillary blood R.B.C. per c.mm.	Venous blood R.B.C. per c.mm.	Capillary blood R.B.C. per c.mm.	Venous blood R.B.C. per c.mm.
4,164,000	4,105,000	3,991,000	3,832,000
Mean difference ... 59,000 Standard error ... ± 38,000 Standard deviation ... 254,000		Mean difference ... 159,000 Standard error ... ± 25,000 Standard deviation ... 155,000	

Standard error of the difference between the mean differences: 46,000.

This difference in the counts in the two groups is capable of two explanations. The diminution in the relative concentration of the capillary blood in the oedematous group may be due to dilution of the blood flowing from the punctured lobe of the ear by excessive tissue fluid. On the other hand, the extra congestion of the veins necessary to secure a sample of venous blood in an oedematous subject may produce some concentration of the blood in the veins. It is impossible to say which of these processes is predominant and, almost certainly both contribute something to the result. If the first suggestion is correct it would be preferable to do all blood counts on venous blood when studying anaemia in nephritis. But the accuracy of individual counts must also be considered since it is with individual counts, during oedema and after it has subsided, that the observations on the degree of anaemia of a patient will be judged subsequently. Examination of the individual counts shows that the venous counts on any one subject have a much wider week-to-week variation than have the capillary counts. This is shown in Table 1 where the standard deviation of the differences in the oedematous group (254,000) is considerably larger than that in the non-oedematous group (155,000).

From these results it appears that in the presence of oedema, blood counts made either from capillary or venous/

venous blood are subject to an unavoidable experimental error. In the former there is some degree of dilution, in the latter, of concentration. In neither case, however, is the error great, and in view of the fact that capillary blood is much more easily obtained and shows less variability than venous, all subsequent counts have been made from blood obtained by skin puncture alone. It must, however, be borne in mind that in capillary blood counts, oedema may produce a false lowering of the red cell count by about 100,000 red blood cells per c.mm. and a proportionate (2.5%) reduction in haemoglobin and other blood constituents.

PART II.

THE EFFECT OF OEDEMA ON THE CONCENTRATION OF RED CELLS IN THE BLOOD STREAM.

The possibility that oedema of the body tissues may also involve the blood-stream and result in an apparent anaemia due to hydraemia has been considered by a number of authorities. Grawitz (1911) was the first to suggest that anaemia in nephritis was due to a simple hydraemic plethora but he found it only in cases of chronic nephritis with congestive heart failure and therefore his hypothesis cannot apply to the common types of oedematous nephritis in children, namely, acute haemorrhagic nephritis and nephrotic nephritis, in neither of which does congestive heart failure commonly occur. Thursfield (1934) observed that acute nephritis with oedema was often accompanied by a marked anaemia and considered that this was more apparent than real as it was in all probability due to oedema of the blood.

McClure and his co-workers (1933) made a detailed study of the red cell count of capillary blood in nephrotic nephritis during phases when oedema was increasing, stationery, and subsiding. They found that as oedema increased, the red cell count, haemoglobin, and packed cell volume rose; when oedema was stationery the counts and haematocrit readings decreased and when oedema was subsiding and/

and there was well marked diuresis the counts and haematocrit readings continued to fall until they reached their original level. They considered that these changes might be explained by movement of fluid from the blood to the tissues during the period of increasing oedema while during lessening oedema, fluid returned from the tissues to the blood and they concluded that in the nephrotic syndrome the fault lies in the tissues rather than in failure of the kidneys to excrete water. In order to find out whether there was any relationship between the red cell count and the stage of the disease in the patients under investigation, a comparison was made between the results obtained at three different stages in patients with acute nephritis and oedema and in those with nephrotic nephritis.

In the former group of twelve patients (Table 3) the red cell count is recorded:

(1) when the patient had well marked oedema, shown by pitting on pressure and subsequently by a sharp fall in weight:

(2) whenever pitting oedema disappeared and diuresis was well marked, and

(3) some 4 or 5 weeks later when water balance was re-established and there was no oedema and no diuresis.

TABLE 3./

TABLE 3.

No.	Name.	Red cells per c.mm.		
		Oedema + or ++	First record when no oedema diuresis + +	4 to 5 weeks later no oedema no diuresis
1	D.F. ...	4,335,000	5,160,000	Irreg. Dismissal
2	A.J. ...	4,025,000	5,130,000	4,010,000
3	E.R. ...	4,755,000	5,115,000	4,440,000
4	W.S. ...	4,205,000	4,635,000	4,325,000
5	R.M. ...	3,985,000	4,375,000	-
6	M.W. ...	3,730,000	4,065,000	-
7	G.C. ...	3,660,000	4,315,000	4,000,000
8	F.C. ...	4,265,000	5,455,000	4,770,000
9	M.M. ...	3,985,000	4,640,000	4,045,000
10	A.R. ...	3,665,000	4,210,000	3,825,000
11	D.T. ...	4,785,000	5,020,000	4,410,000
12	W.H. ...	3,585,000	4,340,000	3,655,000
Averages * ...		4,103,000	4,762,000	4,164,000

(* Excluding No. 1, 5 and 6).

It can be seen at once that during diuresis in acute nephritis there is an increased concentration of red cells in the blood stream. Unfortunately, cases of acute nephritis do not come under observation during the/

the brief initial period when oedema is increasing and there is marked oliguria, otherwise it would probably be possible to demonstrate that there is a reduction in the concentration of red cells during this early phase.

In nephrotic nephritis the changes in the red cell concentration of the peripheral blood with fluctuations in oedema are quite different. The results in Table 4 are examples of changes which were recorded several times for each child, though on some occasions the counts were complicated by the presence of an acute infection which lead to a rapidly advancing anaemia.

TABLE 4.

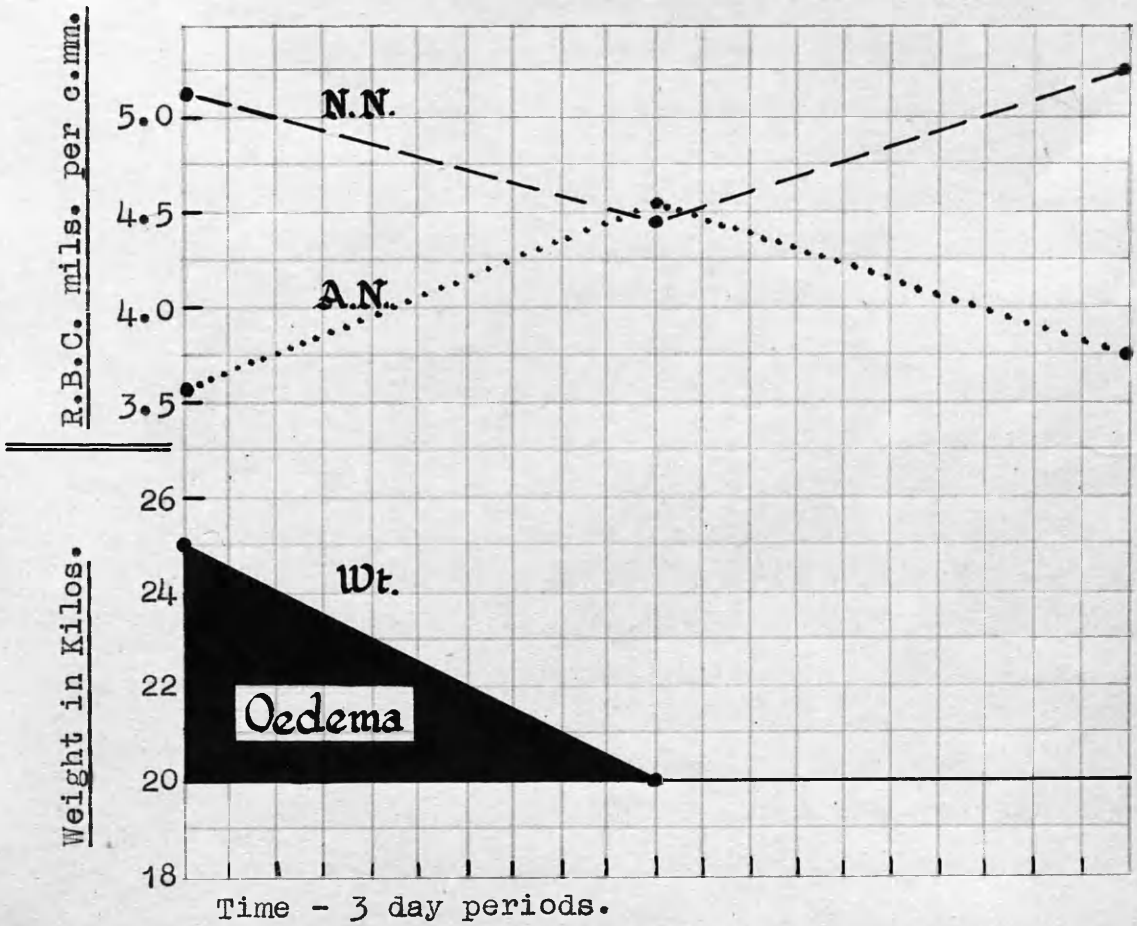
BLOOD AND URINARY FINDINGS DURING PERIODS OF INCREASING AND DIMINISHING OEDEMA.

Name.	Oedema.	Hb per cent.	R.B.C. per c.mm.	Packed Cell Vol. per cent.	N.P.N. MGM. per cent.	Blood Cl. MGM. per cent.	Urine c.c. 24 hr.
J.M.	Increasing	104	5,495,000	47.0	32.4	443	480
	Diminishing	96	4,910,000	40.0	22.7	472	1070
W.G.	Increasing	95	4,815,000	46.3	24.9	410	660
	Diminishing	82	4,110,000	40.0	19.0	484	940
T.M.	Increasing	103	5,640,000	55.1	35.7	432	230
	Diminishing	96	5,225,000	47.0	23.8	462	1250

In the examples shown, there was no evidence of acute infection/

CHART 1.

RED CELL COUNTS DURING AND AFTER OEDEMA.



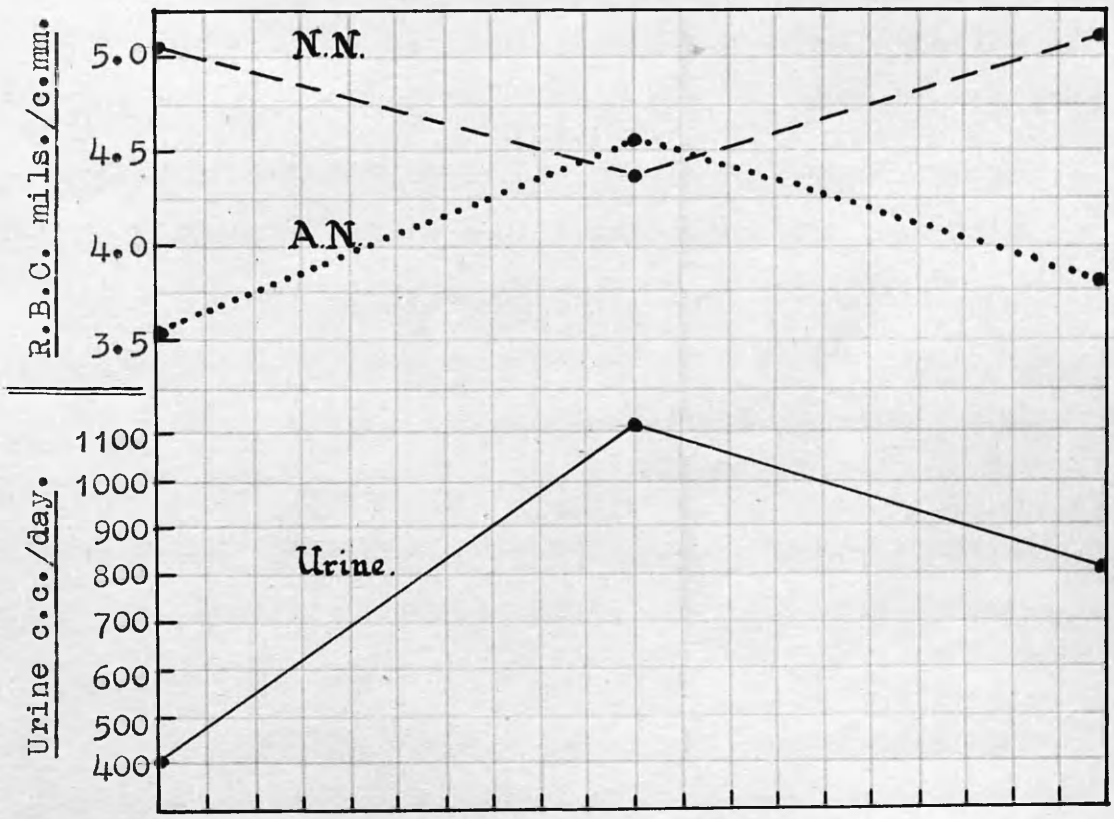
Body weight in Kilos.

Red Cell Count in Nephritis. (Acute)

Red Cell Count in Nephrotic Nephritis.

CHART 2.

RED CELL COUNTS DURING DIURESIS.



Volume of urine excreted in 24 hours.

Red cell count in acute nephritis.

Red cell count in nephrotic nephritis.

and recovery in acute nephritis with oedema is quite different from that in nephrotic nephritis and this will be discussed more fully in the section dealing with plasma volume.

It will be seen that in acute nephritis a series of blood counts done when the patient is recovering and after the first flood of diuresis has passed, will disclose what appears to be an increasing anaemia but is in reality only a diminishing haemoconcentration, and the true red cell count can only be obtained about 4 weeks after oedema, indicated by pitting on pressure, has disappeared.

In nephrotic nephritis any anaemia that may be present will tend to be masked by the presence of increasing oedema with its associated haemoconcentration and the true blood count can be obtained only during a non-oedematous phase when the weight is steady.

The observations of McClure et al. (1933) on nephrotic nephritis are entirely confirmed by the results obtained in this study.

PART III.

ANAEMIA IN NEPHRITIS.

Soon after Bright's (1836) orginal description of nephritis, it became recognised that the disease is sometimes accompanied by a severe and progressive anaemia. Since then, the problems presented by this very intractable anaemia have been extensively studied in adults, but there has been little detailed study of the blood changes occurring in the nephritis of childhood, though at this age period, complicating factors are less common than in adults. Part of the difficulty in assessing these studies arises from a failure on the part of many workers to differentiate the types and stages of the disease before presenting their results and in other cases, where a differentiation has been made, the meaning which the author attaches to his subdivisions of nephritis is too often uncertain.

Parsons and Ekola-Strolberg (1933) have made a careful review of the early literature on anaemia in nephritis and they state that "anaemia is common in chronic nephritis."

Christison (1841) was one of the first to estimate the degree of anaemia in nephritis and he considered that it was a very useful guide to the progress and prognosis of the disease. He devised a method which is to some extent analogous to the modern haematocrit tube. The blood was defibrinated/

defibrinated by whipping and then allowed to sediment and by comparing the proportion of red cells to serum which he found in chronic nephritis, with his results in healthy subjects, it can be calculated that the average red cell count of his patients was 3 to 3.5 million R.B.C. per c.mm.

Leichtenstern (1878) also found considerable anaemia in chronic nephritis. Parsons et al. (1933) after an extensive investigation of the published reports and from the examination of their own cases, concluded that anaemia is almost always found in cases with azotaemia regardless of the pathological basis for the renal insufficiency and further claimed that there is a parallel between the degree of anaemia and the extent of azotaemia, but Townsend et al. (1937) while they found some relationship between anaemia and azotaemia, admitted that some cases with severe azotaemia were not anaemic and vice versa. Wintrobe (1934) studied anaemia in nephritis, most of his cases having the chronic types of the disease, and he found that the anaemia was normocytic or microcytic but never hypochromic. The average red cell count in his series was 3.58 mils. per c.mm. with a haemoglobin of 70% Haldane, colour index of 1.0, packed cell volume of 30% and mean corpuscular volume of 84 cubic microns. The value of this finding of an orthochromic normocytic anaemia is diminished by the inclusion of such different diseases as acute haemorrhagic nephritis and nephrosclerosis in one group.

A/

A somewhat greater degree of agreement is found among the studies reported by those who have differentiated the stages of nephritis.

McLean (1927) considered that in acute haemorrhagic nephritis, almost all cases with oedema and a few cases without oedema "show an increase in the fluid part of the blood so that the percentage of blood solids is less than normal."

Thursfield (1934) claimed that acute nephritis is often accompanied by a marked anaemia but that this was more apparent than real and should be attributed to oedema of the blood.

Cass (1939) stated that anaemia in acute nephritis was slight and tended to spontaneous recovery, while Murphy et al.(1934) practically never found anaemia in mild cases.

Van Slyke,Stillman et al.(1930) found that in the acute stage of haemorrhagic nephritis the temporary anaemia was of no more significance than the temporary fall in blood urea clearance and observed that some patients had slight anaemia while others had none at all.

Boyd (1927) examined some fifty children with acute nephritis and concluded that gross haematuria could produce a secondary anaemia of a severity proportional to the amount of blood lost through the kidney.

It is generally agreed (Van Slyke et al.,1930; Cass, 1939; Bell/

Bell, 1938; Murphy et al.,1938; Leiter, 1930; Fahr, 1937) that in nephrotic nephritis anaemia occurs only in association with an acute septic infection and in these cases it progresses with startling rapidity.

Chronic haemorrhagic nephritis is the type of the disease in which anaemia is found most commonly and in its most severe form. Van Slyke et al. (1930) found it 'practically always present' and in their patients the haemoglobin was usually from 32 to 64% Haldane.

Murphy et al. (1934) claimed that a red cell count below 3.5 mils. per c.mm. implies progressive renal breakdown. Cass (1939) considered anaemia a late complication of chronic haemorrhagic nephritis; in her patients the anaemia was orthochromic and normocytic and nearly always accompanied by nitrogen retention though the degree of anaemia was not related to the extent of nitrogen retention.

Bell (1938) found a hypochromic anaemia of fairly severe degree present in the great majority of cases and the haemoglobin was 50% or less in two thirds of the patients in which it was determined.

Brown & Roth (1922) studied the incidence of anaemia in 187 cases of chronic nephritis and judged 105 of them anaemic with red cell counts below 4.0 mils. per c.mm. They consider that the anaemia develops in steps and stairs, with each fresh exacerbation of the nephritic process so that in between these 'renal breaks' the renal function tests may be normal/

normal while the anaemia remains only to become worse with each fresh renal exacerbation.

Mitchell (1930) perhaps goes rather far when he claims that in practically every reported instance of chronic interstitial nephritis in which the blood was examined, secondary anaemia was found. It is not quite clear what he means by 'secondary' anaemia.

Most authorities agree (Van Slyke, et al., 1930) that when anaemia is present in nephrosclerosis it is less severe and less intractable than in chronic haemorrhagic nephritis. All investigators are agreed that the anaemia which occurs in nephritis is normocytic or microcytic but there is difference of opinion regarding the colour index.

A hyperchromic anaemia has been described with a colour index ranging from 1.6 (Aubertin et al., 1920) to 1.1 (Berg. 1922) but most workers have found a colour index between 0.9 and 1.0 (Parsons et al., 1933; Scarlett, 1929; Ashe, 1929; Wintrobe, 1934).

In some reports a hypochromic anaemia is described with a colour index between 0.8 and 0.9 (Brown & Roth, 1923; Klemperer & Otani, 1931; Bell, 1938) but no study of anaemia in nephritis has disclosed the pronounced hypochromia characteristic of iron deficiency.

This survey of the literature on anaemia in nephritis shows that while all workers are agreed that anaemia is a very common accompaniment of nephritis there are contradictory statements/

statements regarding the type and degree of anaemia and there is no clear indication in which form or forms of renal disease the characteristically intractable anaemia occurs.

By applying the method of study, outlined in the introduction, to 39 cases of nephritis, an endeavour has been made to gain fresh information on these problems.

The patients have been separated into the following groups, each of which is considered independently.

1. Acute haemorrhagic nephritis - 21 cases.
2. Nephrotic nephritis - 5 cases.
3. Nephrosclerosis - 8 cases.
4. Chronic haemorrhagic nephritis- 5 cases.

1. Acute Haemorrhagic Nephritis.

All the patients in this group showed the typical signs and symptoms of the disease. Their illness was characterised by a sudden onset, often after a streptococcal infection, with constitutional symptoms such as headache, vomiting, anorexia, and pain in the back; with two exceptions all of them gave a history of oedema at the onset of the disease and sixteen were still oedematous on admission. All had albuminuria, haematuria and casts in the urine; the blood pressure was above normal in all but three cases. In about half the cases the non-protein nitrogen was above 40 mgms % at the first examination. All except one of the patients were well and had a normal urine within three months of the onset of the disease. In the single exception, nearly seven months elapsed before the urine was free from albumin.

The/

The results of the blood examinations made during the active stage of the disease (Table 5) show that there was a slight orthochromic normocytic anaemia. The average colour index was 0.9 and the mean corpuscular volume was 86 cubic microns. There was also a slight leucocytosis.

As these counts showed the state of the blood only in the early stage of the disease it was possible that anaemia had not then developed but that estimations made sometime later might disclose its subsequent occurrence. Of the twenty-one patients in this group, eight were dismissed from hospital within six weeks of the first counts being made. In the remaining thirteen patients, the result of blood examinations made six to eight weeks after the initial counts revealed (Table 6) that there was no increase in the anaemia and that the leucocytosis had disappeared.

It would appear from these results that early in acute haemorrhagic nephritis there is a slight orthochromic, normocytic anaemia and a slight leucocytosis and that when convalescence is established the anaemia persists unchanged though there is no longer leucocytosis.

TABLE 5.

THE BLOOD IN ACUTE NEPHRITIS.

SOON AFTER ADMISSION TO HOSPITAL.				
Name.	Red Cells per c.mm.	Hb. per cent (Haldane)	White Cells per c.mm.	R.B.C. vol. per cent.
D.F.	4,335,000	80	11,400	40.6
A.J.	4,025,000	72	13,200	35.0
E.R.	4,755,000	74	12,700	-
W.S.	4,205,000	84	22,900	40.4
M.E.	4,735,000	92	7,200	42.9
E.McI.	4,965,000	90	13,300	46.6
I.M.	4,235,000	72	15,200	39.6
R.M.	3,985,000	70	12,500	35.8
G.H.	4,520,000	92	11,900	43.5
H.G.	3,730,000	70	18,500	38.4
M.W.	3,730,000	72	7,300	39.1
G.G.	3,660,000	72	14,800	33.8
F.C.	4,265,000	74	14,200	37.1
E.M.	4,610,000	78	26,200	39.0
E.S.	4,930,000	88	16,300	43.0
M.M.	3,985,000	78	13,900	38.8
F.G.	4,715,000	80	11,600	38.5
A.R.	3,665,000	70	11,600	34.0
D.T.	4,785,000	88	8,200	39.0
W.H.	3,585,000	72	15,000	30.0
M.S.	4,650,000	82	9,800	35.0
Averages	4,289,000	77	13,700	36.7

TABLE 6.

THE BLOOD IN ACUTE NEPHRITIS.

6 to 8 WEEKS AFTER ADMISSION TO HOSPITAL.				
Name.	Red Cells per c.mm.	Hb. per cent. (Haldane)	White Cells per c.mm.	R.B.C. vol. per cent.
E.R.	4,560,000	80	11,100	-
W.S.	4,645,000	93	11,000	-
M.E.	4,540,000	86	8,300	-
E.McI.	4,785,000	90	11,300	46.5
G.H.	3,750,000	78	11,700	36.1
G.G.	4,440,000	90	7,100	44.6
F.C.	5,210,000	90	8,600	-
E.M.	4,135,000	70	9,000	37.8
F.G.	4,525,000	78	8,400	37.2
A.R.	3,520,000	72	8,900	30.0
D.T.	4,410,000	86	5,800	38.0
W.H.	3,800,000	72	8,000	35.0
M.S.	4,050,000	83	4,900	40.5
Averages	4,340,000	82	8,800	38.4

Averages of above group on admission.
(Extracted from Table 5.)

4,390,000	80	13,800	38.3

2. Nephrotic Nephritis.

Although it is generally recognised that anaemia is not a feature of nephrotic nephritis in the absence of acute pyogenic infection there is difficulty in estimating the true state of the blood on account of the variations in the blood count according to the stage of the disease when the examination is made. In Table 7 the red cell count, the haemoglobin percentage, the white cell count, and the packed cell volume in five children with nephrotic nephritis are recorded. These figures represent the state of the blood on admission to hospital but do not show whether true anaemia is present or not, because the effect of oedema, described in Part 11 of this study, and the effect of infection must be taken into consideration. These five children showed the typical features of nephrotic nephritis except that one of them (W.G.) had a tuberculous infection in addition. The course of the blood changes is best seen by reviewing each case individually.

TABLE 7.

THE BLOOD IN THE NEPHROTIC SYNDROME.

Name	Red Cells per c.mm.	Hb. per cent (Haldane)	White cells per c.mm.	R.B.C. vol. per cent.	Reticulo-cytes per cent of R.B.C.
T.M.	5,640,000	103	6,400	55.1	0.9
J.M.	5,495,000	104	13,400	47.0	0.6
W.G.	4,090,000	78	23,500	39.0	1.2
I.D.	3,810,000	66	17,300	32.4	-
E.G.	4,055,000	78	13,900	33.0	1.4
Averages	4,608,000	86	14,900	41.3	1.02

Average colour index 0.93
Average mean corpuscular volume 90 c. microns.

T.M. - age 7 years.

During his first three years the boy had frequent minor chest complaints but thereafter remained healthy until two days before admission to hospital, when his face became puffy and on the following day he had swelling of his face, abdomen, scrotum, sacrum, and legs.

On admission he had anasarca and ascites with oliguria, albuminuria (28 parts Esbach), casts and scanty red cells in the urine. Blood pressure, 122/92 mm. Hg. The Mantoux tuberculin test was negative. Temperature, pulse rate, and respiration rate were normal. Oedema was increasing rapidly. The blood examination showed:

Red/

Red blood cells	5,640,000 per c.mm.
Haemoglobin	103 per cent.
White blood cells	6,400 per c.mm.
Reticulocytes	0.9 per cent
Packed cell volume	50.1%
Serum Protein	5.13 gms. %
Non-protein nitrogen	35.7 mgms. %

After one week he developed erysipelas of the chest wall followed by an empyema and in the course of four weeks his blood picture changed to:

Red blood cells	3,160,000 per c.mm.
Haemoglobin	52 per cent.
White blood cells	23,400 per c.mm.
Reticulocytes	2.6%
Packed cell volume	35.9%
Serum Protein	5.25 gms. %
Non-protein nitrogen	35.2 mgms. %

Following treatment of the empyema, he made a good recovery and four months later there was no oedema, blood pressure 108/80 mm.Hg., no albumin in the urine, the chest appeared to be quite sound, and the blood picture was:

Red blood cells	5,225,000 per c.mm.
Haemoglobin	96 %
White blood cells	8,600 per c.mm.
Reticulocytes	0.9%
Packed cell volume	47.0%
Serum Protein	7.32 gms. %
Non-protein nitrogen	41.7 mgms. %

He remained well for two weeks then had a relapse of nephrotic nephritis and the clinical, biochemical and haematological findings were the same as on his admission to hospital. After about six months of varying degrees of oedema he again developed erysipelas, pneumonia and empyema. Associated with this there was severe anaemia, a leucocytosis reaching/

CHART 3.

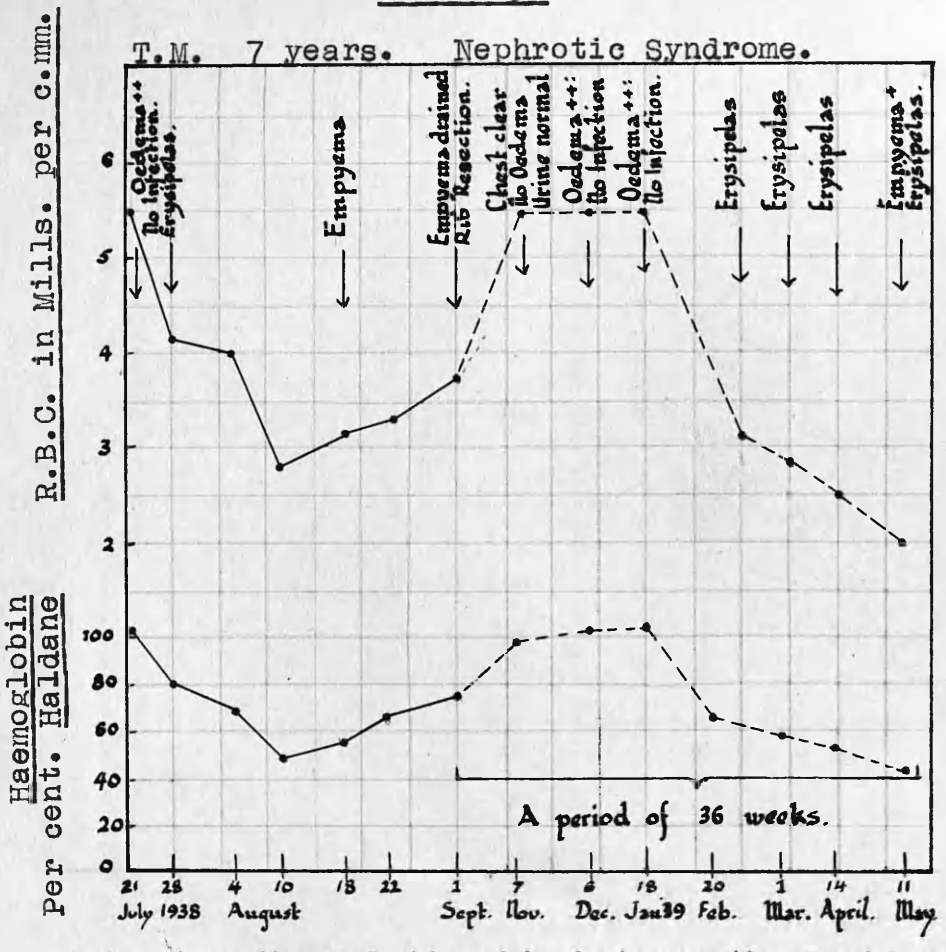


Chart to show the Relationship between the Incidence of Anaemia and Infection in Nephrotic Syndrome.

reaching 46,400 white blood cells per c.mm., and a reticulocytosis varying between 5 per cent and 10 per cent. He died of this infection and 8 days before his death the blood count showed:

Red blood cells	1,825,000 per c.mm.
Haemoglobin	36%
White blood cells	29,000 per c.mm.
Reticulocytes	5.6%
Packed cell volume	24.0%
Serum Protein	5.11 gms. %
Non-protein nitrogen	59.5mgms. %

The course of the anaemia and its relationship to pyogenic infection is shown in Chart 3. The post mortem examination revealed a left sided empyema with a collection of pus under the diaphragm and bilateral pneumonic consolidation. The kidneys presented the features characteristic of lipoid nephrosis.

J. McD. - age 4 years.

The boy had a normal healthy childhood until two weeks before admission to Hospital, when his face became puffy and oedema rapidly spread over his whole body.

On admission there was anasarca and ascites with oliguria.

The urine contained albumin (15 parts Esbach), many casts, but only scanty red blood cells.

The Mantoux tuberculin test was negative, the blood pressure 110/59 mm. Hg. The blood examination showed:

Red/

Red blood cells	5,495,000 per c.mm.
Haemoglobin	104%
White blood cells	13,400 per c.mm.
Reticulocytes	0.6%
Packed cell volume	47.0%
Serum Protein	4.9 gms. %
Non-protein nitrogen	28 mgms. %

Thereafter he spent most of his life in hospital with waxing and waning oedema but no impairment of renal function as tested by urea clearance tests. During one week when he had a sudden increase in oedema, his blood showed red cells 6,185,000 per c.mm., haemoglobin 126% and a week later when oedema was diminishing, the count fell to red cells 4,790,000 per c.mm., haemoglobin 98%. This boy repeatedly showed fluctuations in his red cell count, associated with changes in oedema, similar to the example quoted though less extreme. Some of these fluctuations are represented diagramatically in Chart 4.

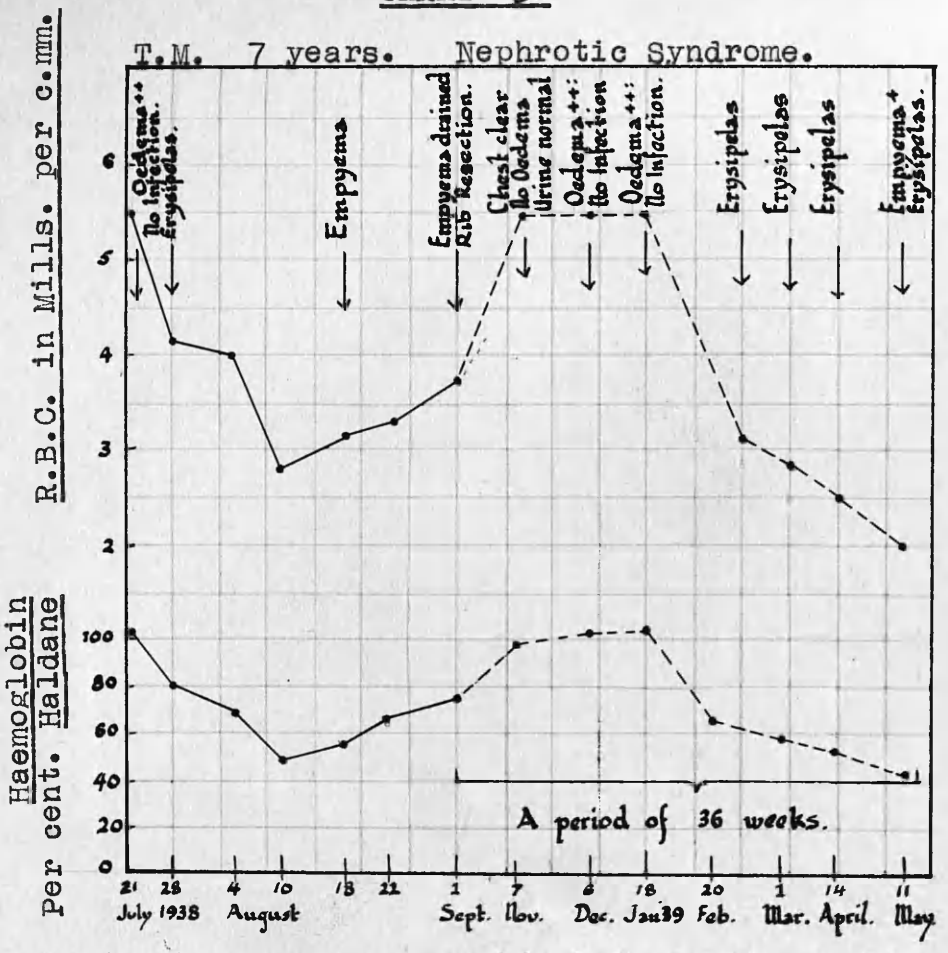
Ultimately, 20 months after the first examination he developed cellulitis of his thighs and abdominal wall and a generalised peritonitis which caused his death.

In the course of this fatal infection he developed a severe anaemia:

Red blood cells	2,785,000 per c.mm.
Haemoglobin	48%
White blood cells	27,300 per c.mm.
Reticulocytes	4.0%
Serum Protein	5.73 gms. %
Non-protein nitrogen	34.2 mgms. %

On post mortem examination he was found to have a generalised pneumococcal peritonitis and both kidneys presented the/

CHART 3.



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Reticulocytes	0.6%
Packed cell volume	47.0%
Serum Protein	4.9 gms. %
Non-protein nitrogen	28 mgms. %

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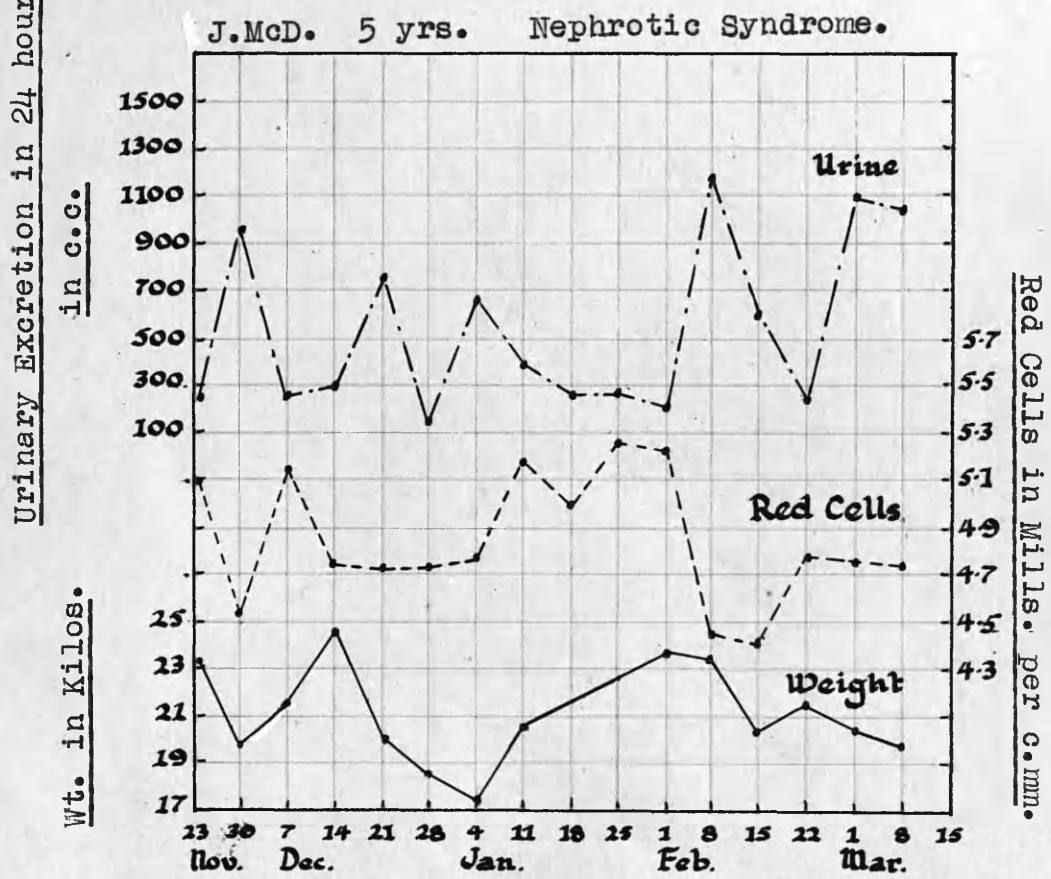


Graph to Show the Relationship Between Red Cell Count, Degree of Oedema and Urinary Secretion in the Nephrotic Syndrome.

the characteristic features of lipoid nephrosis.

W.G. - age 6 years.

This boy had bone tuberculosis at 4 years. During the year before admission to Yorkhill Hospital he spent ten months in two other hospitals with oedematous nephritis.

On admission he had widespread oedema and ascites, gross albuminuria and many casts, but no red cells, in his urine. The Mantoux tuberculin test was positive. Blood pressure 106/70 mm. Hg. The blood findings were:

Red blood cells	4,090,000 per c.mm.
Haemoglobin	78%
White blood cells	23,500 per c.mm.
Reticulocytes	1.2%
Packed cell volume	39.0%
Serum Protein	4.57 gms. %
Non-protein nitrogen	27.8 mgms. %

The oedema gradually disappeared and six months later when he was transferred to another hospital his urine was unchanged but his blood count showed:

Red blood cells	5,190,000 per c.mm.
Haemoglobin	94%
White blood cells	15,300 per c.mm.
Packed cell volume	48.3%
Serum Protein	5.96 gms. %
Non-protein nitrogen	38.6 mgms. %

I.D. - age 4 years.

The girl had a normal childhood. Three days before admission she became listless with anorexia and vomiting and her face was puffy. Two days later her face, legs and abdomen became greatly swollen.

On/

On admission she had anasarca, ascites, and fluid in both pleural cavities. Her urine contained much albumin, a few casts and only occasional red blood cells. The blood pressure was 85/60 mm. Hg. During the next seven months she had several infections and oedema varied from time to time. When the present series of examinations were commenced she was recovering from a respiratory infection but in the course of the next five weeks she developed a generalised peritonitis and died.

The low counts obtained in this patient are attributed to the pyogenic infections from which she suffered. Blood examinations were done on six occasions and each was similar to the first one which recorded:

Red blood cells	3,810,000 per c.mm.
Haemoglobin	66%
White blood cells	17,300 per c.mm.
Packed cell volume	32.4%
Non-protein nitrogen	35.8 mgms. %

E.G. - age 3 years.

The boy had a normal childhood until four days before admission when his face became puffy and the swelling rapidly spread throughout his body.

On admission to hospital he had generalised oedema, the urine contained albumin (20 parts Esbach), numerous casts, but no red cells. Blood pressure 82/58 mm.Hg. The Mantoux tuberculin test was negative.

Blood examination showed:

Red/

Red blood cells	4,055,000 per c.mm.
Haemoglobin	70%
White blood cells	13,900 per c.mm.
Reticulocytes	1.4%
Packed cell volume	33.0%
Serum Protein	5.61 gms. %
Non-protein nitrogen	23.9 mgms. %

Ten days later he was found to have whooping cough and was transferred to another hospital. At that time his count was:

Red blood cells	4,775,000 per c.mm.
Haemoglobin	86%
White blood cells	22,100 per c.mm.
Reticulocytes	1.1%
Packed cell volume	42.0%
Non-protein nitrogen	27.0 mgms. %

It is noticeable in this case with whooping cough that though the infection stimulated a leucocytosis it did not lead to the rapidly increasing anaemia which is so characteristic of pyogenic infections in these patients.

It is claimed that these results justify the statement that anaemia is not usual in nephrotic nephritis in the absence of pyogenic infection but such patients are especially susceptible to these infections and when they do occur, a rapidly progressive and severe normocytic, orthochromic, or slightly hypochromic anaemia results. There is a well marked reticulocytosis and if the pyogenic infection is overcome, regeneration of the blood occurs rapidly in spite of the persistence of nephrotic nephritis.

3. Nephrosclerosis.

Of the eight subjects studied in this section, three (J.D.,/

(J.D., R.L., P.B.) were renal dwarfs and the other five had simple nephrosclerosis. The three dwarfs were typical examples of the disease with severe thirst, polyuria, lassitude and failure to grow. X-rays of their wrists showed the changes characteristic of rickets.

One (J.D.) had a double hydronephrosis and a chronic pyuria from which streptococci were grown. Another (P.B.) had considerable haemorrhage from an injury two weeks before admission to hospital.

Four of the other five cases, (M. McN., J.C., E.D., A.B.) were advanced examples of nephrosclerosis. In each the onset was insidious with headache and vomiting as prominent features and none of them gave any history of an attack of acute nephritis. They never had oedema nor were more than scanty red cells found in their urine during the time they were in hospital. The systolic blood pressure was at some time over 190 mm. Hg. in each case and there was mild albuminuria. All had azotaemia and well marked neuro-retinitis and retinal haemorrhages.

The remaining child (R.M.) was similar to the four cases described above except that she had gross urinary haemorrhage for nearly four weeks before admission. This child had severe anaemia on admission and died eight days later. She had reticulocytosis of 5%, the non-protein nitrogen was 142.8 mgms. % and the serum protein 7.23 gms. %, the/

the packed cell volume was 24.3% and the indirect van den Bergh reaction amounted to only 1 unit. The blood pressure was 190/130 mm. Hg. The child's urine was bright red with fresh blood. The post mortem examination on this girl disclosed a long standing chronic nephritis with a small atrophic right kidney and a larger left kidney. There were developmental abnormalities in the vascular system of the right kidney with stenosis of the right renal artery and the haemorrhage had come from granulations in the pelvis of this kidney.

It was not discovered why the renal dwarf (R.L.) was so very anaemia but as he had a reticulocytosis of 5% and his haemoglobin rose 7% in five days on treatment with ferrous sulphate, it is plain that he was not suffering from the type of anaemia to be described below.

With regard to the other children in this group, reference to Table 8 shows that in these patients, with one exception, no anaemia has been found in the absence of haemorrhage or infection and where these complications existed the blood showed a marked reticulocytosis, and as soon as the haemorrhage was controlled, or the infection overcome, the blood count rose steadily. This sequence of events is well seen in the patient E.D. Her red cell count was over five millions per c.mm. in October and her blood pressure was 204/148 mm.Hg. (Table 9). About three weeks later/

TABLE 8.

THE BLOOD IN NEPHROSCLEROSIS.

Name.	Red cells per c.mm.	Hb. per cent (Haldane)	White cells per c.mm.	Packed cell volume per cent	Notes.
J.D.	4,260,000	74	10,800	40.2	Urinary Infection.
M.McN.	5,415,000	108	7,800	44.7	
J.C.	4,375,000	86	11,700	43.8	
R.M.	2,460,000	50	11,200	24.3	Haemorrhage
E.D.	5,330,000	99	-	41.0	
R.L.	2,205,000	43	6,900	22.0	Cause of anaemia undetermined.
A.B.	5,185,000	100	13,900	51.5	
P.B.	3,890,000	58	10,000	34.1	Injury haemorrhage.

TABLE 9.

THE BLOOD FOLLOWING HAEMORRHAGE IN NEPHROSCLEROSIS.

Name.	Date.	Red cells per c.mm.	Hb. per cent (Haldane)	Packed volume cl. per cent.	White cells per c.mm.	Reticulocytes per cent of R.B.C.
E.D.	16 Oct.	5,330,000	99	41	-	-
"	11 Nov.	2,970,000	58	24	11,800	4.6
"	25 Nov.	4,025,000	80	33	10,800	3.2

later several carious teeth were extracted and during that night she had severe haemorrhage from the tooth sockets and in the course of the next few days her red cell count fell below 3 millions per c.mm. The haemorrhage was controlled with some difficulty. She developed an immediate reticulocyte response of 4 - 5% and within two weeks her blood count was over 4 millions per c.mm. and her haemoglobin 80%. She then left hospital and further examination was impossible.

These observations do not support the contention of Parsons and Ekola - Strolberg (1933) that anaemia and azotaemia are closely related. No anaemia has been found in nephrosclerosis except when haemorrhage or infection occurs as a complication and further, the anaemia that then develops, responds readily to simple treatment.

4. Chronic Haemorrhagic Nephritis.

The five patients in this group were all suffering from a chronic form of nephritis. In four of them there was a clear history of an initial attack of acute nephritis with haematuria, oedema, and constitutional upset. In the other case (P.C.) the illness dated from an attack of pneumonia 8 months before admission. He was known to have albuminuria immediately after the pneumonia.

While in hospital each of these children had albuminuria, haematuria, and numerous casts in their urine.

The/

The systolic blood pressure was moderately increased, usually ranging from 120 to 140 mm. Hg., there was a moderate degree of azotaemia, and oedema that varied in amount from time to time.

The cases in this group are considered to constitute an entirely different disease from nephrosclerosis (van Slyke et al. 1930). They differ from it in that the disease began as an apparently simple acute nephritis which passed into a subacute and chronic stage with haematuria oedema, and moderate hyperpiesia. In nephrosclerosis, on the other hand, the onset is insidious without haematuria or oedema and, except in renal dwarfism, there is a very high blood pressure with associated retinal changes.

Brief notes on each patient indicate the main clinical findings and the characteristic haematology is shown in the records of this group of patients in appendix 1. The records of only four of these patients are recorded in the appendix as the fifth was studied mainly for other purposes, and the haematological findings are included in the text.

P.C. Male aged 7 years.

History: Eight months before admission to hospital he had pneumonia and was in another hospital for two months. Since then he has had persistent albuminuria, his face has been puffy in the mornings and occasionally his feet were swollen at night.

On/

On admission to hospital he had slight oedema, unhealthy tonsils and the blood and urine findings were as follows:

Red blood cells	4,035,000 per c.mm.
White blood cells	11,400 per c.mm.
Haemoglobin	70%
Colour Index	0.9
Packed cell volume	36.4%
Reticulocytes	2.9%
Non-protein nitrogen	41.0 mgms. %
Serum Protein	5.49 gms. %
Blood pressure	134/80 mm. Hg.
Urine: Albumin	4.0 parts Esbach
(24 hrs) Blood	12 million red cells.
Volume	1240 c.c.

Oedema soon disappeared but otherwise his condition remained more or less unchanged. The reticulocyte count varied between 0.6 % and 3.1% but in spite of vigorous treatment with iron, liver, and ascorbic acid, anaemia persisted and four months after the counts recorded above, his blood was almost unchanged. Two years later the boy died at home, his illness having lasted 2 years 8 months.

J.C. Male aged 5 years.

History: Swelling of the abdomen for two years and frequent attacks of tonsillitis. Two weeks before admission his urine was noticed to be dark red in colour and two days before admission he developed anorexia, nausea, and listlessness. On admission he had slight oedema and there was moderate ascites. When first studied, the blood and urine examination gave the following results:

Red blood cells/

Red blood cells	2,440,000 per c.mm.
White blood cells	6,400 per c.mm.
Haemoglobin	44%
Colour Index	0.9
Packed cell volume	25.4%
Reticulocytes	0.6%
Non-protein nitrogen	57.5 mgms. %
Serum Protein	5.09 gms. %
Blood pressure	100/70 mm. Hg.
Urine: Albumin	4.5 parts Esbach
(24 hrs) Blood	400 million red cells
Volume	770 c.c.

Oedema disappeared, but ascites and haematuria persisted, and, though he was slightly less anaemic, his general condition had not improved six weeks later, when he was transferred to another hospital.

A.A. Female aged 8 years.

History: Scarlet fever and ill for six months with kidney disease when 7 years old. Six months before admission her face became puffy and later her legs and arms. Five weeks before admission she had a febrile illness and the swelling became worse and she had headache and vomiting.

On admission she had well marked oedema and moderate haematuria and the state of the blood and urine was as follows:-

Red blood cells/

Red blood cells	3,625,000 per c.mm.
White blood cells	13,600 per c.mm.
Haemoglobin	68%
Colour Index	0.9
Packed cell volume	29.0%
Reticulocytes	1.1%
Non-protein nitrogen	28.7 mgms. %
Serum Protein	5.25 gms. %
Blood pressure	124/78 mm. Hg.
Urine: Albumin	2.0 parts Esbach.
Blood	691 million red cells
Volume	520 c.c.

Oedema slowly disappeared only to come and go later, and the general condition remained more or less stationery. In spite of energetic haematinic therapy in the course of the next six months, the red cell count fell to 2,970,000 per c.mm. and the haemoglobin to 58%.

J.S. Male aged 9 years.

History: Two weeks before admission his face became puffy and he was listless and off his food. One week before admission his urine was noticed to be red and oedema became widespread and severe.

On admission there was general anasarca and ascites, and much blood and albumin in the urine. His blood pressure was 125/75 mm. Hg., non-protein nitrogen was 39 mgms. %, Serum Protein 5.45 gms. %. He developed bilateral pleural effusions but after four months the oedema began to subside gradually. The blood findings were then:

Red/

Red blood cells	3,380,000 per c.mm
White blood cells	9.200 per c.mm.
Haemoglobin	67%
Colour Index	1.0
Packed cell volume	31.0 %
Serum Protein	5.51 gms. %

Three months later all oedema had subsided but his clinical condition was otherwise not greatly changed and he still had albuminuria and haematuria. His blood then showed:

Red blood cells	3,735,000 per c.mm.
White blood cells	14,200 per c.mm.
Haemoglobin	68%
Colour Index	0.9
Packed cell volume	35.0%
Non-protein nitrogen	72.5 mgms. %
Serum Protein	5.96 gms. %
Blood pressure	110/68 mm. Hg.

He was then transferred to a convalescent home where he died about two years later.

J.R. Male aged 7 years.

History: Eighteen months before admission he developed nephritis with oedema and haematuria. The oedema and haematuria cleared up in a few months but albuminuria persisted. One month before admission he became feverish and began vomiting. One week before admission increasing swelling of his abdomen and face was noticed and he was passing very little urine.

On admission he had oedema of the back and legs, ascites and bilateral pleural effusions. His blood pressure was 124/78 mm. Hg. and the urine contained albumin, blood and many granular casts. Diuresis gradually appeared and he soon/

soon lost 7 Kilograms of oedema, but throughout his stay in hospital albuminuria persisted. He was seen several times during the following two years and there was always albumin in the urine. He died at home four years after the onset of the disease.

When the boy was first seen his blood counts gave the following results:

Red blood cells	3,725,000 per c.mm.
White blood cells	6,000 per c.mm.
Haemoglobin	72%
Colour Index	1.0
Packed cell volume	39.0%
Reticulocytes	0.4%
Non-protein nitrogen	24.8 mgms. %
Serum Protein	7.2 gms. %
Blood pressure	124/78 mm. Hg.
Urine: Albumin	0.5 parts Esbach.
Blood	139 mils. red cells.
Volume	410 c.c.

Three months later his blood count was substantially the same.

It is in this group and in this group alone, that the characteristic anaemia of nephritis had been found to occur. The difference between the blood findings in this group (Table 10) and those in the other groups (Tables 5, 6, 7, 8) is striking. In this type of the disease there is considerable anaemia with a slightly greater reduction in the haemoglobin than in the number of red cells, giving a colour index of 0.93. The mean corpuscular volume at 94 cubic microns indicates that the cells are of normal size or slightly larger than normal, a finding in agreement with Townsend, Massie & Lyons (1937). The examinations made about three months later show practically no change in the state of the blood in spite of treatment with iron, vitamin C and liver extract. From Table 10 it will be seen that associated with the anaemia there was a slight leucocytosis. Films showed the red cells well coloured and fairly uniform in size and shape; differential white cell counts on four of these children are recorded in Table 11. No abnormal red or white cells were seen. Three of the children showed a slightly increased proportion of lymphocytes and one had eosinophylia of 3.5%. The number of reticulocytes was less than the degree of anaemia would warrant but never amounted to less than 0.4% of the red cells in any of the 36 counts which were made.

SUMMARY/

TABLE 10.

THE BLOOD IN CHRONIC HAEMORRHAGIC NEPHRITIS.

Soon after admission to Hospital.					Approx. 3 months after admission to Hospital.				
Name.	Red cells mils.per. c.mm.	Hb. % (Haldane)	White cells per c.mm.	R.B.C. vol. %	Name.	Red cells mils. per c.mm.	Hb. % (Haldane)	White cells per c.mm.	R.B.C. vol. %
P.C.	4.035	70	11,400	36.4	P.C.	3.540	64	11,500	33.9
J.C.	2.440	44	6,400	25.4	J.C.	2.990	52	12,200	29.8
A.A.	3.625	68	13,600	29.0	A.A.	3.000	60	8,300	30.0
J.S.	3.380	67	9,200	31.0	J.S.	3.735	68	14,200	35.0
J.R.	3.725	72	6,000	39.0	J.R.	3.730	64	7,740	-
Average.	3.441	64.2	9,320	32.2	Average.	3.399	63.6	10,788	32.2

Average colour index - 0.93

Average mean corpuscular volume - 94 c. microns.

Average colour index - 0.94

Average mean corpuscular volume - 95 c. microns.

TABLE 11.

DIFFERENTIAL COUNTS OF NUCLEATED CELLS IN A GROUP OF CHILDREN WITH CHRONIC HAEMORRHAGIC NEPHRITIS.

Name	Neutrophil Polymorphs	Basophil Polymorphs	Eosinophil Polymorphs	Metamyelocytes	Lymphocytes	Normoblasts	Myelocytes	Reticulocytes Highest	Reticulocytes Lowest
P.C.	47	0	1.0	9	43	0	0	3.1	0.8
J.C.	60	0	0	2	38	0	0	0.6	0.5
A.A.	46	0.5	3.5	5	45	0	0	2.6	0.6
J.R.	75	2.0	0	0	23	0	0	0.6	0.4

Figures all per cent. of all white cells except reticulocytes which are per cent. red cells.

SUMMARY.

Summarising the information obtained regarding the incidence, type, and degree of anaemia found in this series of children with nephritis, the following observations have been made.

(a) In acute haemorrhagic nephritis there is a very slight normocytic orthochromic anaemia which is probably of no special significance.

(b) In nephrotic nephritis anaemia occurs only in association with acute pyogenic infection. When it occurs, the anaemia is normocytic and orthochromic or slightly hypochromic, it develops rapidly, and there is spontaneous recovery from anaemia, if the acute infection is cured.

(c) In nephrosclerosis, anaemia may occur from haemorrhage or pyogenic infection. It is of the same type as in nephrotic nephritis and when the cause is removed spontaneous recovery ensues.

(d) In chronic haemorrhagic nephritis there is a moderately severe normocytic orthochromic anaemia which shows no tendency to spontaneous recovery and is almost entirely uninfluenced by treatment with liver extract, iron and vitamin C. The proportion of reticulocytes is about the normal level and there is a slight leucocytosis.

PART IV.

THE ETIOLOGY OF ANAEMIA IN NEPHRITIS.

The investigations described in this section were concerned mainly with an attempt to elucidate some of the factors involved in the etiology of anaemia in nephritis. Several suggestions have been put forward to explain the development of anaemia in nephritis but none of them has proved conclusive nor contributed anything of value to the treatment of the disease and it is still generally agreed that the cause of anaemia in nephritis remains quite unknown.

The anaemia that is found in nephritis is a normocytic orthochromic anaemia which occurs in the chronic haemorrhagic type of nephritis, is moderately severe, associated with slight leucocytosis, slowly progressive and quite intractable.

On general principles it would seem that there are only four possible causes of the anaemia.

1. Loss of blood from haematuria.
2. Apparent anaemia from blood dilution.
3. Excessive haemolysis of red cells.
4. Defective manufacture of red cells.

An attempt has been made to determine which of these is operative by applying a series of tests designed to show any changes in haemopoiesis indicative of one or other of them.

1. Loss of blood as a result of haematuria.

In every case of nephritis that has been studied, red

cell/

cell counts have been made on the urine as a means of estimating the degree of haematuria and the total amount of blood lost in the urine. The method adopted has been to make a twenty-four hour collection of urine from the patient, then, after thorough mixing, to place a drop of the untreated urine on a haemocytometer and count the number of red cells in one cubic millimetre of urine. From this count the total number of red cells in the whole specimen of urine was calculated. These urinary counts indicated a loss of red cells in the urine varying from none to over 12,000 million per 24 hours.

More than 300 of these counts have been done on 40 patients with some form of nephritis; the counts in most cases were made at weekly intervals. In considering haematuria as a cause of anaemia it has been decided, however, to exclude the twelve patients who were suffering from either nephrotic nephritis or nephrosclerosis, since haematuria is not a feature of these diseases and when anaemia occurs in such patients it is clearly due to some other factor. No very accurate method of showing the results in tabular form is possible but Table 1 gives a rough idea of the relationship between the degree of haematuria at the start of the illness and the extent of the anaemia. The twenty-eight cases under consideration have been divided into three groups; 9 subjects with a haemoglobin level of 70% or less; 8 in whom the haemoglobin/

TABLE 1.

GROUP 1.

Patients with Haemoglobin 70% or less.		
Name	Hb. %	R.B.C. excretion in millions per 24 hours.
R.J.	70	8672
M.R.	60	942
I.M.	66	2380
A.R.	70	2570
J.C.	50	550
H.G.	70	370
A.A.	64	691
E.M.	68	233
W.S.	68	0
9 Average	65	1823

GROUP 2.

Patients with Haemoglobin 71% to 80%		
Name	Hb. %	R.B.C. excretion in millions per 24 hours.
A.D.	80	3019
A.J.	74	3226
G.C.	74	4891
F.G.	80	2718
E.R.	78	638
R.M.	74	840
M.W.	80	455
E.S.	75	20
8 Average	77	1951

GROUP 3.

Patients with Haemoglobin more than 80%		
Name	Hb. %	R.B.C. excretion in millions per 24 hours.
D.F.	98	79
A.M.	86	1943
M.E.	90	1792
G.H.	84	2285
F.C.	92	2050
M.M.	82	4233
D.T.	82	164
W.H.	85	611
M.S.	82	62
P.C.	83	84
E.M.	85	18
11 Average	86	1211

Tables to show a comparison between the degree of anaemia and the severity of haematuria.

The column 'R.B.C. excretion' indicates the number of millions of red blood cells excreted in the urine in 24 hours. The haemoglobin estimations were made about four weeks after the corresponding urinary red cell counts.

haemoglobin was from 71% to 80% and 11 with haemoglobin over 80%. The blood haemoglobin findings selected for comparison with the haematuria were those obtained approximately four weeks after the initial urinary count. This interval was allowed in order to permit time for the haemorrhage to produce anaemia, if indeed it does so. In Table 1 it will be seen that there is no material difference between the loss of red cells in groups "one" and "two" while in group "three" the loss of red cells is rather less. Group "three" included a proportion of patients in whom the disease was mild and who were therefore not sent to hospital as early as the more obviously ill children in the other groups. If they had been studied earlier in the course of the disease the haematuria in group "three" would probably have been equal to that in the other groups. These estimates of haematuria take no account of its duration and are therefore only a very indifferent guide to the total loss of blood which the patients suffered. In Table 2 each of the 237 urinary red cell counts made on these 28 patients, is recorded together with each patient's blood haemoglobin concentrations at the time of the first and of the last urinary count. The urinary counts were usually made at weekly intervals. It will be seen that some patients were studied over a much longer period than others, but this was not always because they were more severely ill.

These/

TABLE 2.

Urinary Red Cell Counts in Millions of R.B.C. Excreted in 24 Hours.

Haemoglobin Concentration of Blood in % Haldane.

Name.	A.D.	A.J.	G.C.	F.G.	E.R.	R.M.	M.W.	E.S.	D.F.	A.M.	M.E.	G.H.	F.C.	M.M.	D.T.	W.H.	M.S.	P.C.	E.M.	R.J.	M.R.	I.M.	A.R.	J.C.	H.G.	A.A.		E.M.	W.S.
1	3019	3226	4891	2718	638	840	455	20	79	1943	1792	2285	2050	4233	164	611	62	84	18	8672	942	2380	2570	550	370	691		233	0
2	2468	706	5280	1704	94	37	144	31	29	1188	815	1178	28	1319	123	382		0	7	7989	4524	468	1655	530	0	511		0	0
3	72	2022	1103	424	17	16	156	0		1020	20	2419	0	141	0	446		12	0	3463	940		1727	400	0	870		21	0
4	194	167	324	619	4					1349	9	1825	0	108	0	112		118	11	4297	696		2204	54	0	1516		2	16
5	45	0	233	494	30					233	0	2160	63	240	13	74		11	0	2086	187		2028	917		937		53	8
6	168	117	1088	595	0					495	0	3027				50		131	7	904	1010		2006	1421		610		21	0
7	0	0	97	106	18					612		3027				6		0	0	1740	1346		1685	2448		281		20	0
8			91		40							701				7		0	13	9480	156		2017			1176		46	
9					0											11		129		5910	1738		1124			490			
10					53													59		10686	4944		925			436			
11																		547		7800	3931		598			360			
12																		189		2096	2604		160			299			
13																		244		1931	2534		167			93	Contd.		
14																		120			846		240			470	211		
15																		104			467		612			164	244		
16																					81		151			202	302		
17																					115		209			232	450		
18																					74		183			232	204		
19																					418		69			843	221		
20																					624		32			207	420		
21																					350		63			144	90		
22																					343					242	82		
23																					339					260	126		
Hb.1)	72	72	72	80	74	70	74	88	80	86	92	92	74	78	88	72	78	70	90	90	42	72	70	44	70	68		78	84
Hb.2)	73	80	90	78	80	74	80	75	98	85	86	78	90	78	82	77		76	90	60	62	66	76	52	70	58		70	93

Hb.1) - Haemoglobin at the time of the first urinary red cell count recorded.
Hb.2) - Haemoglobin at the time of the last urinary red cell count recorded.

These detailed results show that haematuria of considerable degree may continue over many weeks without any fall in blood haemoglobin concentration, but they also reveal that occasionally a long continued haematuria is associated with a falling haemoglobin so that on these grounds, it would be possible that anaemia was due to prolonged loss of blood in the urine. Gladys Boyd (1927) believes that this frequently occurs in acute nephritis in children. With a view to determining whether the blood loss was sufficiently large to account for the anaemia the actual amount of blood lost was calculated from these red cell counts made on 24 hours collections of urine. In this connection it must be borne in mind that on an average, the patient's blood contained only about 4 million R.B.C. per c.mm. A possible fallacy in calculating the blood loss had to be guarded against for if there was haemolysis of the red cells in the urine the red cell counts made would not indicate the total amount of blood excreted and indeed this haemolysis might be more extreme in the very patients who showed the greatest degree of anaemia. In order to gain information on this, two modifications of the same sort of experiment were devised and applied to a few of the children who were being studied.

The first experiment was applied to five children. In three cases with haematuria, specimens of freshly passed urine were obtained and three red cell counts done on each of them/

TABLE 3.

Name.	Method.	First Count: Freshly passed urine R.B.C. per c.mm.	Second Count: After 3 hrs. incubation. R.B.C. per c.mm.	% Loss of R.B.C.	Third Count: After 24 hrs. incubation R.B.C. per c.mm.	% Loss of R.B.C.	Blood Hb. %
A.D.	Urine containing blood	7490	6050	19	2500	53	72
R.J.	Urine containing blood	12820	11710	9	3910	69	72
A.M.	Urine containing blood	4170	3360	19			86
P.C.	Blood added to urine	2160	2000	7	1750	19	80
M.R.	Blood added to urine	8630	6500	25	4530	48	62

Table representing the rate of haemolysis of red cells in urine.

The first three patients had haematuria and the last two patients had blood withdrawn from a vein and added to their urine.

TABLE 4.

Haemolysins in urine of patients with Nephritis.

Name.	Hb. %	Red Blood Cells in Millions per c.mm.	TEST TUBES. 1	2	3	4	5	6
W.G.	92	4.915	-	$\frac{-}{+}$	-	-	-	-
E.M.	94	4.820	-	+	+	$\frac{-}{+}$	-	-
J.M.	95	4.660	-	+	+	$\frac{-}{+}$	-	-
A.M.	86	4.650	-	+	+	+	-	-
A.D.	84	4.630	-	+	+	+	-	-
G.H.	86	4.460	-	++	++	+	$\frac{-}{+}$	-
T.M.	82	4.307	-	+	$\frac{-}{+}$	$\frac{-}{+}$	$\frac{-}{+}$	-
M.E.	86	4.185	-	+	+	+	$\frac{-}{+}$	-
I.M.	66	4.035	-	+	+	$\frac{-}{+}$	-	-
R.M.	74	3.870	-	+	+	+	$\frac{-}{+}$	-
P.C.	70	3.770	-	+	+	$\frac{-}{+}$	-	-
M.W.	72	3.730	-	+	+	+	+	-
J.C.	54	3.295	-	++	++	++	+	+
A.A.	58	3.010	-	+	$\frac{-}{+}$	-	-	-
M.R.	55	2.640	-	+	$\frac{-}{+}$	$\frac{-}{+}$	-	-

++ = Complete haemolysis.
\+ = Well marked haemolysis.
$\frac{-}{+}$ = Trace of haemolysis.
\- = No haemolysis.

The amount of haemolysis after adding varying quantities of urine to a suspension of red cells in saline.

of haemolysins present in the urine of the patients with anaemia than in those who were not anaemic. An average of 0.41 c.c. of urine produced haemolysis in the anaemic group, whereas 0.47 c.c. was required in the group without anaemia. This result does not confirm Brown & Roth's observation that the urine of non-anaemic subjects contains more haemolysins than that of the patients with anaemia and the results are so close that there is no significant evidence of difference between the two groups of patients.

It is considered that the method outlined first, in which counts of the red cells are made, is probably more reliable and more accurate than Brown & Roth's method in which naked eye estimations of haemolysis and the more or less rough quantitative way of making the red cell suspension must lead to considerable experimental error.

Generally speaking, it appears from Table 3 that up to 25% of the red cells are haemolysed in the first three hours and up to 70% in twenty-four hours. Now the routine red cell counts done on the urine in this study were all made on twenty-four hour collections of urine and the counts were not made until about three hours after the collection was completed. Thus it may be assumed that up to 50% of the red cells were haemolysed by the time the collection was completed, and up to about 60% before the counts were made. From this, it may be deduced that the actual loss of blood from these patients does/

does not exceed 3 or 4 c.c. per day at the height of a severe haematuria. In one patient with severe and long continued haematuria it was calculated that he lost approximately 110 c.c. of blood in his urine in the course of 8 weeks. So large a loss of blood as this, is, however, very rare and it cannot be considered possible that the loss of blood in the urine as calculated by this method could account for the true and typical anaemia of nephritis.

Brown & Roth (1922) estimated that the blood loss in chronic nephritis was up to 1.5 c.c. per litre of urine.

Finally it has been shown (Whitby & Brittain, 1946) that chronic loss of blood, such as occurs from bleeding haemorrhoids or in menorrhagia results in a microcytic and markedly hypochromic anaemia which responds readily to iron medication. None of these features occurs in the anaemia of chronic haemorrhagic nephritis.

2. Apparent Anaemia from Blood Dilution.

Reference has already been made to the observations of others that anaemia occurs in association with the oedema of congestive cardiac failure or with other forms of oedema and that this is only an apparent anaemia due to hydraemic plethora. The investigations described in Part II of this thesis provide very strong evidence in support of the hypothesis that changes in the fluid content of the blood do occur in acute nephritis during the period of diuresis and in nephrotic nephritis throughout the oedematous cycle and final proof will be given in the section dealing with plasma volume. Unless these characteristic fluctuations are recognised, anaemia may certainly appear to be present when in fact there is no diminution in the total number of circulating red cells but the effect of hydraemia on the blood practically never produces a change in the red cell count of more than one million red blood corpuscles per c.mm. and cannot therefore account for the severe form of anaemia that occurs in chronic haemorrhagic nephritis. Further, in the severe cases of anaemia in nephritis in children, there is often no oedema and no cardiac decompensation and in these subjects, the anaemia cannot be attributed to hydraemia. Blood volume estimations (Part V) on these anaemic patients also reveal that there is an absolute diminution in the total number of circulating red cells.

Thus/

Thus while diminishing blood concentration produces a steadily falling red cell count for four or five weeks after the peak of diuresis as acute haemorrhagic nephritis subsides, and while the return of the excess of fluid in the tissues to the blood stream during subsiding oedema in nephrotic nephritis leads to a fall in the red cell count yet in the production of the typical and severe anaemia of chronic haemorrhagic nephritis, changes in the water content of the blood play no consequential part.

3. Excessive Haemolysis of Red Cells.

With a view to determining whether this was a cause of the anaemia of nephritis, special attention was directed to certain characteristic signs found in the haemolytic anaemias.

These were:-

1. Jaundice.
2. Reticulocytosis.
3. The indirect Van den Berg Reaction.
4. Excessive urobilinuria.
5. Increased fragility of the red cells.
6. Deficient anti-haemolytic factors in the blood stream.

Particular attention has been paid to the examination of the conjunctivae and skin in each of the 42 patients with haemorrhagic nephritis studied in this connection and in none of them was any jaundice seen though one patient was known to have been jaundiced a few weeks before coming under observation. This one exception was a girl aged 5 years with a severe haemorrhagic nephritis, who developed a catarrhal jaundice early/

early in the course of the disease. She made a good and rapid recovery from the jaundice though the nephritis passed into the chronic stage. Her serum did not show any agglutination with the spirokaete of Weil's disease.

126 reticulocyte counts were done on these 42 patients and varied from 0.1% to 14% with an average of 1.7%. The high level of 14% occurred in the girl who had jaundice referred to above. In no case was there the sustained reticulocytosis so characteristic of haemolytic anaemia and where counts above normal were obtained, the subject was either very anaemic or was undergoing vigorous haematinic therapy.

The Van den Bergh reaction was done at frequent intervals, usually every fortnight, on 41 of these children. 173 of these tests were done and gave the following results:-

Indirect negative	- 151
0.5 units	- 15
1.0 units	- 6
2.0 units	- 1
over 2.0 units	- 0
Total	- 173

The 22 positive results were scattered among 18 different patients, most of whom gave a negative indirect Van den Bergh reaction on several other occasions. Since a reaction below 2.0 units is of no significance, it may be assumed that there is no excess of bilirubin in the serum in patients with haemorrhagic nephritis. The girl referred to above/

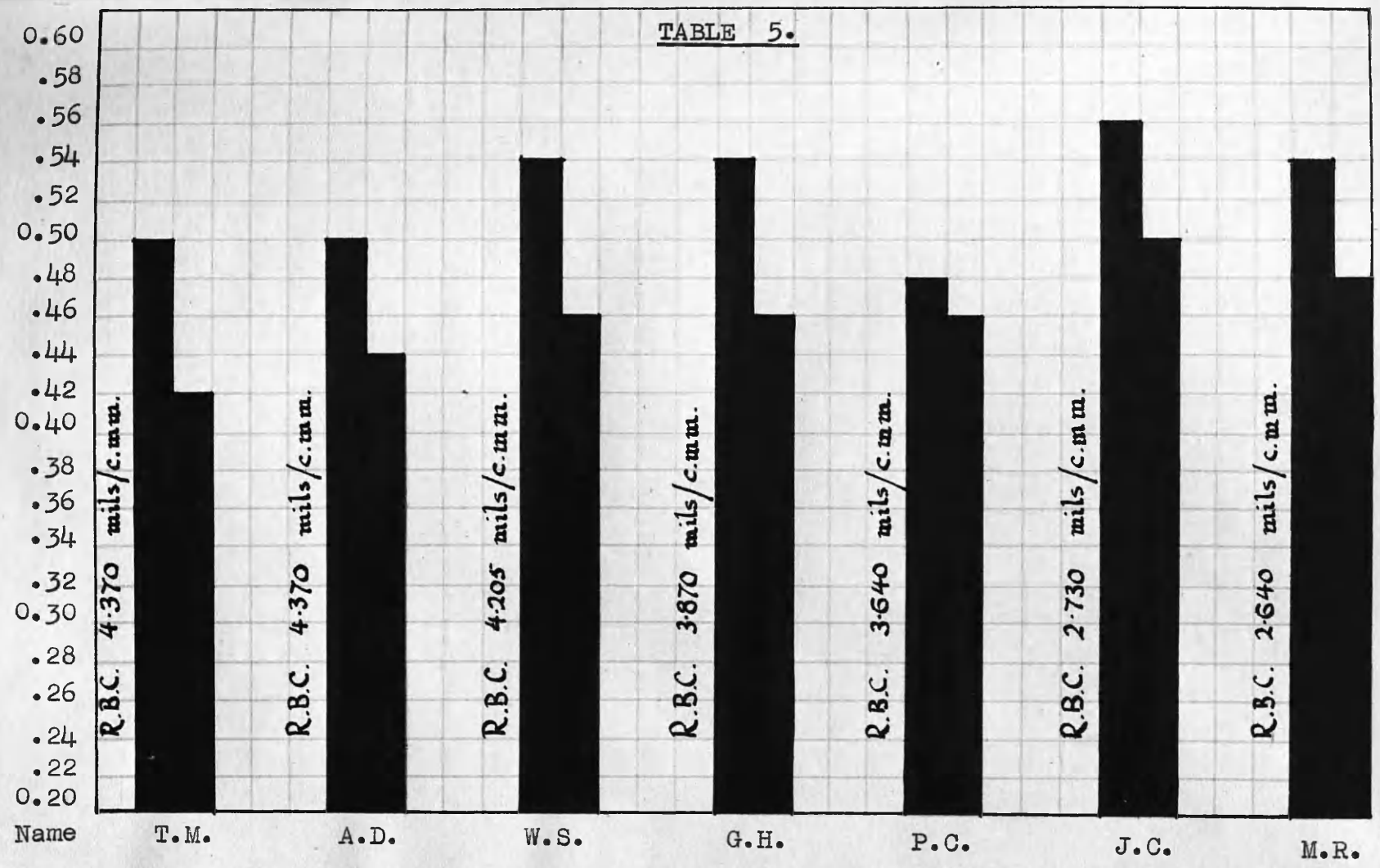
above who had jaundice was not under observation during the course of her jaundice and accordingly, the positive results that would have been obtained do not appear in these records.

The presence of excessive amounts of urobilin and urobilinogen in the urine was first sought for by Schlesinger's test and subsequently this was replaced by Wallace & Diamond's (1925) modification of Ehrlich's diazo reaction. One of these tests was done on a specimen of a 24 hour collection of urine at weekly intervals on all the children. 294 tests were made and in no case did a test reveal an excess of urobilinogen sufficient to be considered pathological, i.e. in a 1/20 dilution, though 6 tests showed a lesser degree of increase over the normal urobilinogen content of the urine and these were attributed to severe haematuria. In this connection it is worthy of note that Brown & Roth (1922) estimated the quantity of urobilin and urobilinogen in the duodenal juice of patients with nephritis and found that it was much diminished both in those with anaemia and in those without anaemia.

The red cell fragility was estimated by the routine qualitative method on 7 subjects with various types of nephritis and with various degrees of anaemia but in every case, the fragility of the red cells was within normal limits and agreed closely with the controls done at the same time.

Clark and Evans (1920) devised a means of estimating the anti-haemolytic effect of the blood serum. They mixed a known/

known volume of washed red cells with a dilute solution of sodium oleate, which acted as a haemolysing agent, and a measured volume of fresh serum, and in this way tested the protective power of the serum against haemolysis in various diseases. They found that the protection against haemolysis afforded by the serum of normal persons and of patients suffering from a wide range of diseases without anaemia, is very constant; that there is some diminution in the protective power in secondary anaemia but that the diminution is most marked in haemolytic anaemias. Brown & Roth (1922) modified this test by doing a duplicate fragility test and adding 0.1 c.c. of fresh serum from the patient to one of the series of tubes and thus found the degree of protection against lysis in hypotonic saline afforded by the serum. They found that the protective power of the serum in a non-anaemic group of patients with nephritis was greater than the protection afforded by the serum of a group with anaemia and nephritis but considered that the difference was not significant. Brown & Roth's test has been repeated with slight modifications of technique, on 7 patients (Table 5). In this small group there were three patients with red cell counts over four millions per c.mm. and four with counts under four millions per c.mm. The average red cell count of the former group was 4.315 million red blood corpuscles per c.mm. and their serum protected the red cells on an average against an increased hypotonicty of 0.073%/



The resistence of the red cells to haemolysis before (Left hand column) and after (Right hand column) adding 0.1 c.c. of the patient's serum to the saline solution.

0.073% NaCl, while in the four anaemic cases the average count was 3.220 million red blood corpuscles per c.mm. and their serum afforded protection against an increased hypotonicity of 0.55% NaCl.

The results of this experiment are in agreement with the findings of Brown & Roth, though we agree with the latter that it is very questionable if the difference between anaemic and non-anaemic groups is sufficient to be significant.

Reviewing the results of all these tests it appears that there is little or no evidence to support the suggestion that the anaemia of nephritis is due to exaggerated haemolysis of red cells.

4. Defective Manufacture of Red Cells.

For long, this explanation has held the field as the most probable cause for the anaemia of nephritis and is the one that calls for most consideration. So far, however, attempts to find a cause for defective manufacture of red cells have proved entirely fruitless. Ceconi (1905) was the first to suggest that the anaemia was due to a bone marrow aplasia of toxic origin. After studying a large series of patients with chronic nephritis, Brown & Roth (1922) concluded that an unknown toxic agent is the most probable cause of both the anaemia and the nephritis. They considered that the anaemia develops in steps and stairs with each fresh exacerbation of the nephritis process so that between these "renal breaks" the renal function tests may be normal while the/

the anaemia remains and becomes worse with each fresh renal exacerbation. They attempted to correlate anaemia with other manifestations of nephritis in an effort to find some etiological factor but without success.

There is no correlation between loss of albumin in the urine and anaemia since the latter is not a marked feature of nephrotic nephritis, a condition in which there is massive albuminuria.

From a review of the literature and from their own extensive researches, Parsons and Ekola-Strolberg (1933) concluded that there is a close parallel between anaemia and azotaemia. This has not been confirmed by other workers (Brown & Roth, 1922) though some (Townsend, Massie & Lyons, 1937, Cass, 1939) claim that anaemia is nearly always accompanied by nitrogen retention but acknowledge that the degree of anaemia is not related to the extent of azotaemia. Brown & Roth (1922) thought that there might be some relationship between increased blood creatinin and anaemia but admitted that such a relationship was by no means invariable and severe anaemia can occur with a normal blood creatinin.

Diet as an etiological factor must have serious consideration in a disease in which the routine treatment often adopted may involve considerable restriction in this respect. It is known (Brown & Roth, 1922) that fasting, low caloric, and low protein diets do not in themselves produce anaemia/

anaemia in healthy human subjects but it has to be admitted that care must be exercised in attributing the same conclusions to subjects who are suffering from disease. It has been shown (Weech, Wollstein & Goetsch,1937) that dogs fed on a low protein diet may lose more than 50% of their red cell volume in 90 days.

Mettnier, Minot & Townsend (1930) observed that nutritional anaemia was much more liable to develop, and much more rapid in its course, when associated with arteriosclerosis. The more severe anaemias in the present study have been associated in every case with a moderate hyperpiesia of long duration. The systolic blood pressure usually varied between 110 mm. Hg. and 140 mm. Hg. On the other hand, it has already been noted that anaemia does not occur in uncomplicated nephrosclerosis in which the blood pressure is much higher and the dietetic restrictions are of a similar nature. Vaughan (1938) stated that achlorhydria is often found in the anaemia associated with nephritis and that this defect favours the development of anaemia. Townsend, Massie & Lyons (1937) have made a detailed study of the relationship between the anaemia of 'chronic glomerulo-nephritis' and gastric acidity. They studied 48 adult cases of chronic glomerulo-nephritis and noted a moderately severe orthochromic normocytic anaemia associated in 60% of the cases with hypochlorhydria after an alcohol test meal. Nineteen of these patients had a red cell count below 4 millions per c.mm./

c.mm. and all of them had hypochlorhydria, five having absolute achlorhydria even after histamine stimulation. Of the 27 patient with red cell counts over 4 millions, 22 had normal gastric acidity. In Townsend, Massie & Lyon's series, the diminution of gastric acidity and the degree of anaemia were found to be proportionate and they concluded that "one of the most important features in the anaemia of chronic glomerulo-nephritis is the diminished or absent hydrochloric acid in the gastric secretion." In an endeavour to confirm in children this important observation in adults, 20 gastric acidity tests have been done on 18 of the patients in this series. The ordinary Reyfuss fractional test meal using strained gruel, and without histamine stimulation has been employed, and the detailed results are recorded in Appendix 2.

The results obtained are summarised in Table 6. Taking 20 - 40 c.c. N/10 HCl. as the normal maximum range for HCl secretion in childhood (Ogilvie, 1934), in 8 of the tests, or 40%, there was hypochlorhydria. In one of these there was complete achlorhydria and in another two, only traces of free acid too small to be estimated quantitatively were found. Of the others, 7 showed normal secretion and 5 hyperchlorhydria. Unfortunately, only 2 cases with severe anaemia were tested and though neither of these showed hypochlorhydria it is possible that, if a larger number of very anaemic patients had been examined, hypochlorhydria would have been found as commonly/

commonly as in the less anaemic patients.

TABLE 6.

THE GASTRIC ACIDITY IN PATIENTS WITH NEPHRITIS.

Red cell count of blood per c.mm.	No. of tests.	Average of maximum figures.		No. of individual tests in each class.		
		H.Cl.	Total acids.	Hypochlor-hydria.	Normal	Hyperchlor-hydria
4 millions and over.	9	29	49	4	2	3
3 to 4 millions	9	25	50	4	3	2
Less than 3 millions	2	37	51	0	2	0
Average of all tests.	20	28	50	8	7	5

Though the results are not nearly as striking as those of Townsend, Massie & Lyons, they do show that in a considerable proportion of cases of nephritis, there is hypochlorhydria. They do not, however, confirm that this bears any definite relationship to the degree of anaemia. This investigation revealed no constant relationship between hypochlorhydria and the presence of anaemia.

There has been much discussion of the relationship between nephritic anaemia and aplastic anaemia and it has been suggested by some workers (Brown & Roth,1922; Ceconi, 1905; Parsons/

Parsons et al.1933) that in nephritis there is some unknown toxic agent which causes a bone marrow aplasia. There is, however, very good evidence against the hypothesis that the anaemia of nephritis is analogous to idiopathic aplastic anaemia.

Isaacs (1937) has published the results of some excellent studies of the bone marrow obtained by sternal puncture during life and post-mortem in various anaemias. In primary idiopathic aplastic anaemia he found that the block in maturation occurred at the primitive 'blast' group of cells and both the 'blast' cells and the subsequent forms were very scanty. By contrast, in the anaemia of nephritis, while the block occurred still at the 'blast' group, the primitive undifferentiated 'blast' cells were very much increased in number and subsequent forms were scanty showing a condition similar to that seen in untreated pernicious anaemia. Isaacs considered that this state of the marrow in chronic nephritis with anaemia is quite characteristic. He further found that the sternal marrow in chronic infection shows a block at the normoblastic stage, which is again totally different from that in nephritis.

Townsend, Massie & Lyons (1937) also examined the bone marrow of 31 cases dying of nephritis and also found it hyperplastic and not aplastic but they were of the opinion that the hyperplasia was of the normoblastic type.

Further/

Further it may be noted that idiopathic aplastic anaemia is associated with leucopenia since the white cells are affected as well as the red cells, whereas the severe anaemia in the patients described here was accompanied by a slight leucocytosis.

SUMMARY OF PART IV.

The investigations that have been described lead to certain conclusions regarding the cause of anaemia in nephritis.

Haematuria may, in exceptional cases, be so severe that it contributes materially to the production and maintenance of some degree of anaemia. It is probable, however, that the anaemia so produced is not the characteristic anaemia of chronic haemorrhagic nephritis and haematuria cannot account for the anaemia in the great majority of cases. Variations in the fluid content of the blood occur at well defined stages in acute haemorrhagic nephritis and in nephrotic nephritis. These changes may simulate true anaemia and if their presence is overlooked, may lead to an erroneous diagnosis, but they very rarely produce a variation in the blood count of more than 1,000,000 red blood cells per c.mm. and play no part in the production of the true anaemia.

All the evidence is against haemolysis as an etiological factor in the anaemia of nephritis.

Of all the causes of all forms of anaemia defective manufacture/

manufacture of red cells is by far the commonest and it is probable that the anaemia of nephritis must also be attributed to some condition detrimental to the development of the red cell.

No correlation can be shown between anaemia and azotaemia or any other pathological feature of nephritis and no toxin has ever been demonstrated in the blood of these patients.

There is some resemblance between this type of anaemia and the anaemia that occurs in chronic sepsis. In both there is a very hyperplastic state of the bone marrow and no diminution of reticulocytes in contrast to the markedly aplastic marrow and absence of reticulocytes so characteristic of idiopathic primary aplastic anaemia. The excessive accumulation of primitive cells and the absence of maturer forms in the bone marrow in nephritic anaemia suggests by analogy with other types of anaemia, that some factor necessary for maturation is deficient. Whether it is rendered inactive by circulating toxins or is quantitatively inadequate as a result of dietetic or other insufficiency cannot as yet be decided. It may be added that in the course of the present study, vigorous treatment of a number of the more severely anaemic patients with large doses of Vitamin C, iron, and liver, has confirmed the long recognised fact that these substances have no curative effect whatsoever.

PART V.

THE BLOOD AND PLASMA VOLUME IN NEPHRITIS.

It has been shown (Part 11) that in certain types of nephritis, serial red cell counts and haemoglobin estimations done during the course of the disease revealed fluctuations which were closely related to the degree and course of oedema and to the volume of urinary excretion. During the phase of recovery in acute nephritis, with the onset of diuresis and the consequent diminution in oedema, the blood count rises by about 700,000 red blood cells per c.mm. and when the excess of tissue fluid has been excreted and diuresis subsides the red cell count returns to its previous level. In contrast, in the nephrotic syndrome the red cell count rises with increasing oedema and oliguria and falls sharply during diminishing oedema and diuresis. The haemoglobin shows similar and proportionate changes. These characteristic fluctuations suggest that the mechanism of diuresis and recovery in acute nephritis with oedema is quite different from that in the chronic nephrotic type. Changes in the red cell count, haemoglobin content, and plasma - red cell ratio, however, do not necessarily indicate changes in plasma volume for they may be brought about by alteration in the total number of circulating red cells. An attempt has been made to determine whether the observed changes were in fact, due to variation in the plasma volume in these types of nephritis/

nephritis and plasma volume estimations have been done at different stages of the disease in 12 patients with acute nephritis and 5 patients with nephrotic nephritis. In addition, the blood volume was estimated in 5 patients with nephrosclerosis and in 6 cases of chronic haemorrhagic nephritis, for only by direct measurement of the blood and plasma volumes can decisive evidence regarding the presence of anaemia be obtained.

Blood Volume Methods.

In 1854, H. Welcker described the first successful attempt to measure the amount of blood in the body. His experiments were carried out on animals and his method consisted in opening a vein on either side of the body. Physiological saline was allowed to flow into one vein at about the same rate as the blood flowed from the other. This was continued until the heart stopped beating when a pressure system was applied and saline forced through the circulation until the fluid flowing from the open vein was perfectly clear. The perfusion was then stopped, samples of the tissues were macerated and their haemoglobin content estimated. This haemoglobin was then added to the haemoglobin obtained by perfusion and the total haemoglobin of the body was thus measured. The blood volume was then calculated from the concentration of haemoglobin in the blood before commencing the bleeding.

Bischoff/

Bischoff (1856) subsequently applied the same technique on two condemned criminals. This method indicated a total blood volume of about 1/13th of the body weight but has been criticised on the grounds that it includes myohaemoglobin which is not part of the circulating blood.

Welcker's method has been modified by numerous workers who introduced various refinements of technique and one of the most useful of these modified methods is that of Harris (1920): his experiment may be divided into three stages:-

1. A 1 c.c. sample of blood is withdrawn for haemoglobin estimation.

2. Bleeding is commenced and at the same time, gum saline is infused to a vein on the opposite side of the body and this is continued until the animal shows signs of collapse when both bleeding and infusion are stopped.

3. A further five minutes is allowed for the blood still in the vessels to mix thoroughly with the gum saline and then another 1 c.c. sample is withdrawn and its haemoglobin content estimated.

When the haemoglobin of the last sample is subtracted from the haemoglobin of the first sample, taken as 100%, it indicates what percentage of the blood has been withdrawn and the actual quantity can be calculated readily from the volume and haemoglobin content of the perfused fluid. This method gives a blood volume of about 1/14th (7%) of the body weight.

The second method of blood volume estimation to be developed was dependent on the affinity of haemoglobin for carbon monoxide. Grehant and Quinquaud (1882) originated the method/

method and it was later adopted and improved in this country by Haldane & Smith (1899) so that it could be applied to human subjects. It involves considerable technical difficulties but is said to be perfectly safe in health and disease, and, in the hands of experts, to have an experimental error of only 5% (Arnold et al.,1921; Barcroft & Stephens, 1927). The principle of the method consists of the introduction of a known quantity of carbon monoxide to the blood through the lungs and the subsequent estimation of the amount of the gas in a known volume of blood. This method indicates a blood volume in adult humans of between 1/16th and 1/17th of the body weight (Salvesen, 1919), much lower than that found by other methods. However, the method shows a high degree of accuracy when applied before and after controlled haemorrhage and by doing repeated daily estimations (Arnold, 1921). Criticism of this type of experiment has been mainly directed to alleged failure of the carbon monoxide to reach relatively stagnant parts of the circulation during the allotted time (Barcroft, 1925) and to the inaccuracy of the haematocrit as a measure of the red cell - plasma ratio throughout the body (Roberts & Crandal, 1933; Smith et al.,1921).

A third method of estimating blood volume was introduced in 1915 by Keith, Rowntree and Geraghty and is really an estimation of the circulating plasma volume from which the blood volume is calculated by using the haematocrit. In/

In this method, a foreign substance is introduced directly into the blood stream, some time allowed for the substance to become evenly distributed throughout the plasma, and then a sample of blood withdrawn and the concentration of the injected substance in it estimated colorimetrically or by other means. The difficulty in this method was to find a substance having all the necessary characteristics.

1. Readily miscible with the plasma.
2. Not excreted from the circulation within the time necessary for complete mixing.
3. Easily recognisable in the blood sample.
4. Non-toxic.

Keith, Rowntree & Geraghty (1915) used a rare dye called 'vital red' in their earlier experiments but later changed to 'congo red' for reasons that will be discussed below. Apart from these two dyes many other substances have been used in attempts to avoid various limitations of the method.

Dawson, Evans & Whipple (1920) tested a very large number of different dyes and found that many of them gave results almost identical to the original vital red. They preferred a special blue azo dye (T 1824) as being easier for colorimetric reading.

Seeking a substance which would remain entirely within the circulation, Meek & Gasser (1918) used gum acacia in/

in blood volume experiments in rabbits and obtained good results. The method is considered undesirable because it is necessary to inject such a large amount of gum acacia that it must itself cause considerable change in the blood volume and further, the method of estimating the concentration of the substance in the blood is not very accurate.

In a similar attempt to find a substance that would be non-toxic and would remain within the vascular system for some time, Lee & Whipple (1921) tried haemoglobin, with satisfactory results, in dogs. The advantage claimed was that though the haemoglobin was entirely eliminated from the blood stream in 18 hours, it was removed very slowly at first. The method has not found general favour because accidental haemolysis in taking a blood sample cannot be recognised, suitable haemoglobin is very difficult to prepare, and the method has no advantage over the original dye methods.

The injection of a known quantity of anti-toxin or of whole blood has also been tried (Lamson & Nagayama, 1920). The former method is unsuitable and inaccurate as it requires biological assaye of the anti-toxin, but the latter method, though less accurate than the dye injections, may give a useful result and is easily applied when a blood transfusion is done for therapeutic purposes.

Ashby (1925) suggested that the intravenous injection of group 'O' blood to a recipient of one of the other groups could/

could be made use of as a means of estimating the blood volume by a process of differential agglutination of the cells in the recipient's blood shortly after the transfusion. He described a technique for carrying out this differential agglutination and thereby estimating the dilution of the donor's cells in the recipient's blood.

The method has been developed by McMichael et al. (1943) who claimed an experimental error of only ± 5%. The method is only of use in anaemic patients and is subject to the grave danger of unrecognisable variations in the plasma volume of the patient during the comparatively bulky transfusion.

With the recent introduction of radio-active elements to clinical practice, attempts have been made to utilise some of these substances for blood volume estimations.

Hahn et al. (1940) have described a method whereby radio-active iron is united with the red blood cells and remains firmly attached to them for several days. The activation can be done only 'in vivo' and as this involves activating the whole of the donor's blood, the method can not be used extensively in human beings. More recently a technique has been developed (Hahn et al,1942) for estimating red cell volume after feeding anaemic dogs with radio-active iron, but radio-active iron is very difficult to prepare and can have no important place in routine human blood volume experiments until more of the technical dangers have been overcome. However it has been shown that radio-active Phosphorus can be prepared/

prepared and attached to red cells 'in vitro' (Hevesy et al., 1944) and the activated red cells can be injected into human subjects. The technical difficulties and unavoidable experimental errors of all these methods depending upon radio-active elements renders them of little or no value in routine experimental practice. Their main value has been to show conclusively that mixing of the injected material in the blood stream is complete within less than three minutes and that no consequential reservoir of red cells exists outside the active circulation in the blood vessels (Hahn et al., 1942).

Went & Drinker (1929) developed a micro-method in which the result is obtained by matching a capillary tube against prepared standards. This method was used on cats, rabbits, guinea pigs and mice with apparent success. It has not been used in human beings as it cannot be as accurate as the results obtained with a colorimeter.

It is commonly agreed that the Welcker and the carbon monoxide methods give the correct red cell volume and the dye method gives a relieable plasma volume (Smith et al., 1921), and even if the absolute values are not accurate there is certainly no doubt that the carbon monoxide and dye methods provide accurate means of measuring changes in blood volume (Hopper et al., 1944). Of these three basic methods, it is at once obvious that the Welcker method cannot be used in clinical practice, since it sacrifices the life of the subject. The

carbon/

carbon monoxide method has proved very accurate in the hands of some experts (Arnold et al.,1921; Salvesen,1919; McIntosh, 1929; Waterfield,1931) but others have found considerable variations in its results, perhaps due in part to the great number of technical difficulties involved in the method. Further, the results obtained are nearly always much lower than those of any other methods.

The dye method has much less technical difficulty, gives fairly consistent results, and is well suited for clinical application (Lamson & Nagayama,1920). It was, therefore, decided to use one of the modifications of Keith, Rowntree & Geraghty's original 'vital red' method.

Working on this method in London, Harris (1920) was striving to find some dye that would be red in the blood, so as not to give the subject a peculiar colour after injection, and yet could be altered to another colour after removal from the body and so avoid any possible confusion with accidental haemolysis. He tried congo red, which is an indicator that is red in the form of its sodium salt and blue in the form of a free acid. The dye proved a failure for the purpose for which it was tried as it was not sufficiently concentrated in the serum to give a suitable change of colour on acidifying, but Harris considered that for blood volume estimations it was a more suitable dye than vital red because it is -

1. commercially available.

2./

2. Less Rapidly excreted from the blood.
3. Non-toxic.
4. Produces less physiological change in the subject.

Rowntree & Brown (1929) tested congo red and finding it entirely satisfactory used it instead of vital red in all their subsequent experiments. Lindhard (1926) on the other hand tested congo red but found that it changed its shade on mixture with the plasma proteins and he discarded it in favour of vital red.

Dawson et al.(1920) claimed that vital red, congo red, and the blue dye T. 1824, were equally efficacious and others (Robinow & Hamilton, 1940; Gregersen, 1938) have confirmed their work. Recently, however, a number of workers have favoured the blue dye, T. 1824 (Schultz et al., 1940; Evans & Gibson, 1937; Davis, 1942; Gregersen, 1944; Walters et al., 1947). The blue dye has the disadvantage that a higher concentration is required in the blood to give a suitable colour for matching, and it often gives the patient a very unhealthy appearance. On the other hand, it has a different absorption spectrum from haemoglobin and so an estimation can be made in the presence of slight haemolysis when a spectrophotometer is used for the colorimetry. It has no advantage over the earlier dye, congo red, unless the colorimetry is done with a spectrophotometer (Gregersen, 1938) and after extensive trials with both spectrophotometer and compensating colorimeter for the dye method of plasma volume estimation, Weech et al.(1937) preferred/

preferred the compensating colorimeter as being just as accurate as the spectrophotometer, and much less time consuming.

In the present series of tests it was decided to commence with Keith, Rowntree & Geraghty's method using congo red and a Bürker compensating colorimeter and as the procedure proved entirely satisfactory it has been used exclusively.

The Dye Method of Keith, Rowntree & Geraghty and its limitations.

Keith, Rowntree & Geraghty's (1915) method of estimating the circulating blood volume consists of the injection of a known quantity of 1% congo red (vital red in the original experiments) into an elbow vein of one arm after an 8 c.c. sample of blood has been withdrawn. Exactly 4 minutes after the completion of the injection a further 8 c.c. sample of blood is withdrawn from the elbow vein of the opposite arm. Both blood samples are added to a suitable anti-coagulant and centrifuged until packing of the red cells is complete. The first sample provides undyed plasma for use as a compensating fluid in the subsequent colorimetry and the second sample gives the haematocrit reading and the dyed plasma. A standard solution of the dye is made up with distilled water: the dyed plasma and water are then compared with the undyed plasma and the standard aqueous solution of the dye in a suitable compensating colorimeter. From the figures so obtained the degree of dilution of the dye in the plasma/

plasma can be deduced and the circulating plasma volume can be calculated. By applying the haematocrit observation the total volume of the blood is calculated.

When properly carried out, this method gives an experimental error of only ∓ 5% (Keith, Rowntree & Geraghty, 1915) and in experienced hands only half of that (Lindhard, 1926). It is essential that a compensating device be used in the colorimetrical examination as the crystalloids and colloids in the plasma alter the optical absorption of the dye (Graff & Clarke, 1931).

At this stage it is advantageous to examine some of the technical and physiological limitations of the method outlined above.

The entire estimation should be done on a fasting subject in order to obtain plasma as free from lipoids as possible.

During the first minute or so the dye is said to pass from the circulation more rapidly than subsequently (Rowntree & Brown, 1929) and from 5% to 8% passes out of the circulation during the first hour (Gregersen, Gibson & Stead, 1935). It is generally agreed that after the first six minutes the dye disappears from the blood at about the rate of 2.5% to 3% per hour (Graff et al., 1931; Gibson & Evans, (1) 1937) or rather faster (Hooper et al., 1920) and passes into the intercellular spaces where it is slowly taken up by the reticulo-endothelial cells/

cells (Graff et al.,1931). It is excreted in the urine and into the bowel and congo red has been demonstrated in the thoracic duct and in the ureters of dogs in 7 minutes and 11 minutes respectively after its injection (Harris, 1920). Almost 50% of the dye is excreted in the bile (Smith, 1930).

Serial estimations show that the dye rapidly becomes evenly mixed throughout the circulation. According to Graff et al. (1931) it takes at least 6 minutes for this to become complete but Rowntree & Brown (1929) in a large series, found no difference in dye concentration at 3, 4, and 6 minutes. It seems likely that the change in dye concentration between 3 and 6 minutes is within the experimental error of the method and most workers have adopted 4 minutes as the best interval between the dye injection and taking the 2nd sample of blood.

In order to find out the true initial dilution of the dye, Gibson & Evans (1) (1937) recommend taking serial samples of blood, after injection of the dye, and thus getting a curve of the disappearance of the dye from the circulation and then by extrapolation of this curve backwards to the zero time they calculate what they call the true dilution. This procedure requires about 100 c.c. of blood and is therefore not applicable in children and in any case the experimental error of the method does not justify such a correction (Graff, D'Esopo & Tillman, 1931). Although all these experiments, designed to discover the ideal time for taking the/

the second sample of blood, have been done on adults, the same interval, namely, 4 minutes, has been adopted in the present study of children, because the rate of mixing of the dye is dependent on the circulation rate (Gibson & Evans, (2) (1937) and since this is more rapid in children than in adults (Seckel, 1936) it is obvious that if mixing of the dye in adults is complete in 4 minutes it will certainly be so in children.

A certain amount of stasis is essential, especially in oedematous children, in order to get a needle into a vein and to secure the blood samples. This should, however, be of as short duration as possible, and if the stasis is not maintained for more than one minute, the error produced is probably not significant (Bennett et al., 1938). The amount of fluid lost by 100 c.c. of blood held in the vessels for 30 minutes at 80 mm. Hg. is only twenty cc.s. (Landis, 1937) or 2/3 c.c. per minute. It is possible however, that the rate is increased in certain oedematous subjects.

After withdrawing the blood samples, Keith, Rowntree & Geraghty originally used finely powdered potassium oxalate as the anti-coagulant but Hooper et al. (1920) showed that dry oxalate caused a shrinkage of the red cells of from 3% to 10% depending upon how much oxalate was used. They recommended the use of 1.6% isotonic neutral potassium oxalate which they found by theory and experiment was satisfactory in the proportions of 2 c.c. of this oxalate solution to 8 c.c. of blood. The/

The originators of this method acknowledged and confirmed this improvement in their technique and they adopted it in all subsequent estimations (Brown & Rowntree, 1929). Curiously enough, it was long overlooked, and is still overlooked by some workers, that Hooper's experiments were conducted on dogs blood and Graff & Clark (1931) repeated the anti-coagulant tests on human blood. They found that 0.9% neutral potassium oxalate resulted in the smallest degree of haemolysis and 1.1% oxalate in the smallest degree of crenation. In a few cases, however, 1.0% oxalate also cause slight haemolysis and so they adopted 2 c.c. of 1.1% neutral potassium oxalate to 8 - 10 c.c. of blood as the ideal method of preventing coagulation.

In calculating the proportion of plasma in the haematocrit tube, and the dilution of the dye in the plasma, allowance must naturally be made for the dilution of the plasma by the oxalate solution. Immediately after withdrawal the blood is transferred to a 15 c.c. haematocrit tube containing the prepared oxalate solution, covered with a thin layer of liquid paraffin to prevent evaporation and the red cells are maximally packed by centrifugalising at 2,500 revs/min. for thirty minutes (Hooper et al., 1920).

The dye can be injected rapidly and the amount injected has no effect on the result. (Keith, Rowntree & Geraghty, 1915; Dawson et al.,1920) 1 c.c. of 1.0% congo red/

red per 5 Kilograms of body weight gives a suitable concentration. A suitable standard solution of congo red must be made up with distilled water so that a reading near the middle of the colorimeter scale is obtained, as certain inaccuracies arise if the standard and the unknown are not reasonably near the same colour. In the present study of children 4 c.c. of 1.0% congo red has been injected in every case and a 1/400 solution of congo red has proved a suitable standard solution in almost every case although occasionally a more dilute solution is required where there is a high plasma volume in an older child. The standard should be made up afresh on each occasion as the colour soon fades.

By doing experiments in vitro with human blood, and using a Burker compensating colorimeter, Lindhard (1926-76) found that the experimental error should not exceed $\pm$ 5.0% and in experienced hands should not be more than half as great.

Not more than seven blood volume estimations can be made on one subject with accuracy as the dye is too rapidly removed from the circulation thereafter (Lindhard,1926-77).

In an attempt to slow down the rate of excretion of the dye, efforts have been made to block the reticulo-endothelial system by previous injections of the same dye or of indian ink, but without success (Smith,1930; Reeve & Armin,1946).

Recently,/

Recently, a method has been developed in which the dye, (T.1824) is extracted from the serum before being quantitatively estimated (Crooke & Morris, 1942) but the results obtained are no more accurate than the older methods and the laborious technique has therefore no advantage (Reeve & Armin, 1946).

From the dye method an estimate is made of the plasma volume, while the haematocrit reading gives the ratio of red cells to plasma and from these data the total blood volume may be calculated. On the reliability of these two estimations depends the accuracy of this method of determining the blood volume. As regards the haematocrit readings, Smith, Arnold & Whipple (1921) suggested that the red cell - plasma ratio in the veins was not a true index of the state of affairs in the general circulation and they claimed that in the capillaries, there is a central stream of fast moving red cells surrounded by a much larger collar of relatively slowly moving plasma. They estimated that about a third of the contents of the small vessels is comparatively stagnant and that about a third of the total blood volume is in these vessels. The small vessels would therefore contain a greater proportion of plasma than the larger veins. They believed that it was on account of these conditions that there is so large a discrepancy between the blood volume calculated by the carbon monoxide method and by the dye method and they suggested/

suggested that the former method gives low results and the latter too high results. These workers found that haematocrit determinations on various arteries and veins gave the same values, but because of the disproportion in the capillaries they concluded that the true blood volume could be obtained only by estimating the red cell volume by the carbon monoxide method and the plasma volume by the dye method and adding together the two volumes so obtained.

Rowntree & Brown (1929) did not consider that this was at all necessary and asserted that there was no conclusive evidence of different concentration of red cells in different parts of the circulating blood. They took synchronous samples of blood from similar veins in each arm and got almost identical values. Taking samples from veins in an arm and a leg gave slightly different results because the leg veins were smaller and it took much longer to secure the sample of blood. They were convinced that the haematocrit afforded an accurate indication of the red cell - plasma ratio in the general circulation.

Walters (1934) was unable to demonstrate any difference in haematocrit estimations done by taking ten consecutive samples of blood from one vein with the point of the needle moved to different positions in it. He concluded that the cells were evenly distributed in the veins.

Fahraeus (1929) was the first to demonstrate the truth of the hypothesis of Smith, Arnold and Whipple and he showed that/

that the blood in the smaller vessels and capillaries contains fewer cells per unit volume than that in the larger vessels. By applying the fundamental principles of engineering dynamics to the circulation through the blood vessels it has been shown (Hahn et al. 1942) that, on theoretical as well as practical grounds, there must be a layer of relatively stagnant plasma along the walls of the blood vessels and an axial stream of cells and plasma and that the proportion of this layer of slow moving plasma to the diameter of the vessel must be greater in small vessels than in large ones.

The work of Landis (1937) on the capillaries has also shown that there is an axial stream of red cells surrounded by relatively stagnant plasma and he observed that when the flow of blood through a capillary is stopped by gentle compression, the corpuscles are at first distributed uniformly throughout the capillary and if the filtration and absorption pressures are balanced, the corpuscles retain this distribution indefinitely. If the capillary pressure is high, the red cells become concentrated, and if low, they become diluted by tissue fluid.

In the investigations described in Part 1 of this thesis it was found that red cell counts on capillary blood obtained by puncturing the lobe of the ear gave a considerably higher figure than counts done simultaneously on venous blood and/

and on first thoughts this might appear to be incompatible with the observations described above. The explanation is as follows. When a capillary is cut across, the blood that flows from the cut end contains a much higher concentration of cells than the blood in the intact capillary because the relatively stagnant cuff of plasma in the vessel, having little or no motion, does not flow from the cut end, and so only the concentrated axial stream of blood escapes (Fahraeus and Lindquist,1931). When blood is aspirated from a large vein, the aspirating force withdraws not only the axial stream but also the cuff of slowly moving plasma and therefore blood obtained by aspiration of a vein should always be less concentrated than blood flowing from a severed capillary.

In adults, moderate occlusion of the arm veins has been found to make no difference to the plasma-corpuscular ratio (Bennet et al.,1938).

Recent work on the distribution of red cells throughout the vascular system appears to indicate that the haematocrit value for venous blood may be about 20% higher than that of the circulation as a whole owing to the lower red cell concentration in the minute vessels (Wright,1945; Hahn et al.,1942; Hevesy et al.,1944), and therefore it is probable that the red cell volume when calculated from the haematocrit and the dye plasma volume is about 20% too high (Davis,1942) and the total blood volume is about 9% too high (Hevesy/

(Hevesy et al.,1944). This source of error in the absolute values is probably invariable in all patients and so does not vitiate comparisons between the blood volume of different patients or of the same patient at different times. In other words, the dye method remains an accurate means of measuring changes in blood and plasma volumes (Hopper et al., 1944).

The haematocrit, when used with all the appropriate precautions, has probably an experimental error of less than 0.5 per cent and is therefore more accurate than red cell counts or haemoglobin estimations (Wintrobe, 1933) and Wintrobe considers that it constitutes the most useful single criterion of the degree of anaemia at present available.

The Normal Blood Volume and Some Factors Influencing It.

It is readily recognised that the blood has no fixed volume, for fluid is constantly passing between the vessels and the tissue spaces as the needs of the organism require and at the same time new red and white cells are being formed and old ones destroyed. During health, these processes are more or less balanced but many different circumstances may lead to one of them becoming temporarily exaggerated. There is probably a large reservoir of blood in the liver, spleen, intestines, and subpapillary plexus of the skin (Seckel, 1936) which may increase greatly the circulating blood volume at times. Barcroft (1925,/

(1925, 1927) has shown the great importance of the spleen as a blood storing organ in rabbits, cats and dogs, but in man, the spleen contains much less muscular tissue and is probably of small importance in the regulation of the blood volume (Wright, 1945; Ebert & Stead, 1941).

The normal blood volume has been ascertained for all animals commonly used for experimental purposes and human beings. Only rabbits and guman beings have been used in the experiments to be described and the latter have been sub-divided into adults and children

1. The Blood Volume in Rabbits.

It can be seen from Table 1 that there are wide variations in the results obtained by workers using different methods and the discrepancy between the dye and carbon monoxide methods is very plain. The gum acacia method of Meek & Gasser (1918) is considered unsatisfactory because it involves bulky injections which must themselves affect the blood volume and the accuracy of the method of assaying the acacia in the blood has not had sufficient confirmation.

The micro-method of Went & Drinker (1929) was specially designed for use in small animals and requires only 0.01 c.c. of blood which is drawn into a capillary glass tube and matched against a series of known dilutions of the dye in the same way as pH is estimated by the capillator technique.

2./

TABLE 1.

THE BLOOD VOLUME OF HEALTHY MALE RABBITS.

Reference.	Number of Experiments.	Average Weight Kg.	Plasma Volume ml/Kg.	Blood Volume ml/Kg.	Method.
Meek & Gasser(1918)	7	-	-	54	Gum acacia
Salvesen (1919)	11	2.255	-	50	Carbon Monoxide
McQuarrie & Davis (1920)	4	3.119	-	74	Dye
Went & Drinker (1929)	-	-	-	87	micro-dye.
Nice & Katz (1934)	16	2.228	58	90	Dye

2. The Blood Volume in Man.

Table 2 summarise the results obtained by a number of workers using different methods and studying normal healthy adults. Generally speaking the carbon monoxide method gives very low results and the red dyes give results that are accurate at least for the plasma volume. Blue dyes nearly always indicate a value somewhat lower than red dyes. It is clear that there is considerable variation in the blood volume of normal subjects and it is important to determine the limits of normality. Bock (1921) believed that the plasma volume was very constantly about 51 ml. per Kg. of body weight. Keith, Rowntree & Geraghty (1915) in their original communication gave the limits of plasma volume in the normal man as 42 to 56 ml. per Kg. (1/23rd to 1/17th) of body weight and the blood volume as 78 to 97 ml. per Kg. (1/13th to 1/10.5th) of body weight. Rowntree & Brown (1929) altered this somewhat and provided the standard commonly accepted at present.

Blood Volume 70 - 100 ml. per Kg. body weight. Average 88 ml/Kg.
2550 -4010 ml. per Sq. M. of surface area.

Plasma Volume 40- 60 ml. per Kg. body weight. Average 53 ml/Kg.
1425 -2250 ml. per Sq. M. of surface area.

Gibson & Evans (1937) considered that the relationship of blood volume to surface area was not more constant than to body weight. This, however, is not the general opinion.

Brown/

TABLE 2.

THE BLOOD VOLUME OF HEALTHY MEN.

Authority.	Number of Experiments.	Age.	Weight; Kg.	Haemoglobin (% Haldane)	Haematocrit Packed cell volume; %.	PLASMA			WHOLE BLOOD			Method
						Total ml.	ml./Kg.	ml/Sq.M.	Total ml.	ml/Kg	ml./Sq.M	
Salvesen (1919)	6	Adult	65.4	117	-	-	-	-	3888	59	-	Carbon Monoxide
Bock (1921)	5	"	67.0	119	-	3414	51	-	5468	82	-	Vital Red.
Brown & Rowntree (1929)	74	"	-	-	-	-	51	1920	-	88	3278	Congo Red.
Gibson & Evans (1937)	49	"	68.6	-	44.7	2948	43	1614	5335	78	2931	T.1824
Davis (1942)	11	"	65.9	-	-	2680	40.5	1538	5071	77	2897	T.1824
Hopper et al(1944)	9	"	-	-	-	-	45.5	-	-	80.5	-	T.1824

Brown & Rowntree (1929) were able to confirm the observation of Dreyer and Ray (1910) that variations in the blood and plasma volumes are less marked when expressed in terms of surface area than of body weight, and they concluded that "it would seem that the blood volume in relation to body surface must be one of the most constant features of the human body."

There are not sufficient data available to prescribe the limits of day to day variations in the blood volume of an individual person, but the general effect of certain factors has been described.

Age. (Table 3).

There is much less agreement about the normal standards of blood volume in children and no really comprehensive investigation has yet been published. Dreyer and Ray (1910) have shown by exsanguination experiments that in mammals, small young animals have a relatively greater blood volume than larger and older animals of the same species. By analogy it may be assumed that the blood volume of children, in relation to their weight, is greater than that of adults.

Robinow & Hamilton (1940) observed that the mixing of the dye in the circulation of infants was complete in 90 seconds. This is to be expected because the circulation time in infants is 14 - 15 seconds (by the histamine rash method) whereas in adults it is 23 - 30 seconds (Seckel, 1936).

TABLE 3.

THE PLASMA AND BLOOD VOLUME OF NORMAL INFANTS AND CHILDREN.

Authority.	Number of Estim-ations.	Age Group.	Plasma Volume ml./Kg.	BLOOD VOLUME.	
				ml./Kg.	Total ml.
Bakwin & Rivkin (1924)	25	< 1 yr.	61 (38 to 72)	101 (71 to 148)	
Lucas & Dearling (1921)	30	<15 dys.	59 (42 to 77)	147 (107 to 195)	
	11	< 1 yr.	67 (57 to 78)	109 (90 to 126)	
Darrow, Soule, Buckman (1928)	52	(Birth	50		
		(6 mths.	62		
		(4 yrs.	50		
Robinow & Hamilton (1940)	20	Newborn		98	
McIntosh (1929)	10	2 mths. to 21 mths.			
Brines, Gibson, Kunkel. (1941)	9	Birth			300
		1 yr.			600
	41	Puberty			2500
Schlutz, Morse, Cassels, IOB, (1940)	7	14 yrs.	45		
	9	17 yrs.	47		
Seckel (1936)	?	Child-hood	50	83 (73 to 93)	

Considerable changes must take place in the blood volume during the neo-natal period with the rapid fall in haemoglobin and in Lucas and Dearlings (1921) series of 30 infants under 2 weeks old, the blood volume varied between 107 c.c. per kg. and 195 c.c. per kg. while the haemoglobin was from 75% to 135%. It is doubtful whether mean figures obtained by including all these infants in one group serves any useful purpose.

Darrow et al. (1928) observed that the plasma volume is low at birth, rises rapidly during the first 6 weeks and thereafter falls until it reaches the normal adult level at 4 years. The study by Schultz et al. (1940) included in Table 3, was made on negro boys.

Present knowledge suggests that the blood volume in relation to weight is about 100 ml. per Kg. during most of infancy and thereafter gradual decreases to reach the adult level of 88 ml. per Kg. at about 4 years of age. The blood volume decreases after middle age (Gibson & Evans, 1937).

Sex. (Rowntree & Brown, 1929; Gibson & Evans, 1937)

Plasma volume in terms of weight or surface area is the same in men and women but blood volume is 7 to 14% greater in men. The difference between the sexes is found almost entirely in the red cell component. The sex difference begins to appear at puberty (Brines et al., 1941). Pregnancy is always associated with an increased plsma volume.

Nutrition./

Nutrition. (Keith, Rowntree & Geraghty, 1915).

Obesity results in a relatively low blood volume as much of the body weight is due to comparatively avascular adipose tissue. For the contrary reason, emaciation is associated with a relatively high blood volume. Marasmic infants have been found to have slightly increased plasma and blood volumes in relation to their weight (Bakwin et al., 1924), although if they are dehydrated, the plasma volume is usually reduced (McIntosh et al.,1930).

These characteristics are related to muscular development and body form and therefore athletic women have blood volumes similar to normal men and sedentary men have blood volumes similar to normal women (Gibson & Evans, 1937).

Emotion. (Nice & Katz,1934; Freeman,1933).

Nice & Katz found that during excitement the blood volume of rabbits remained about constant though there was a withdrawal of plasma from active circulation with compensating increased volume of red cells. Freeman (1933) also found this but he considered that if the sympathetic stimulation was continued for some time the red cells also became reduced so that ultimately there was a diminished blood volume.

Exercise, Heat, Feeding.

Seckel (1936) considered that movement, feeding and heat increase the blood volume and he assumed that this occurred/

occurred by emptying of the blood stores into active circulation. He estimated these stores at 30 or 40 ml. per Kg. in children or about 1/3rd of the normal resting blood volume.

On the other hand Rowntree & Brown (1929) claimed that the blood volume was somewhat diminished by heat.

In the course of their celebrated expedition to the Peruvian Andes, Barcroft and his colleagues (1923) noted that a sustained warm atmosphere, either in the tropics or in a hot chamber, produced a gradual rise in the blood volume amounting, in adults, to about one litre. They were uncertain regarding the effect of altitude because the temperature changes were so great. Bazett (1938) confirmed Barcroft's observation and in an experiment in which two men lived in a room kept at 74.5°F the blood volume was increased 6% in one day, 10% after two days and 25% after five days. He also found that the blood volume in midsummer in America was 30 - 40% higher than in mid-winter. His conclusions were that mild heat raises the blood volume whereas extreme heat lowers the blood volume through sweating and other means.

Kaltrieder and Meneely (1940) demonstrated that exercise decreased the plasma and blood volumes but the effect on the red cell volume was variable.

Thompson et al. (1928) have shown that the erect posture greatly prolongs the time required for the dye to become/

become evenly distributed throughout the circulation and they claimed that standing caused a reduction of 11% in the plasma volume and that the reduction was maximal in 20 to 30 minutes. Exercise produced the same change but more rapidly. The blood volume is closely related to the Basal Metabolic Rate and therefore to the oxygen requirements of the organism.

Blood Pressure. (Keith, Rowntree & Geraghty, 1915).

No striking changes were found in the blood or plasma volumes associated with fairly marked variations in blood pressure. Collapse, whether toxic or reflex, results in a low blood and plasma volume (Seckel, 1936).

Polycythaemia. (Seckel, 1936; Brown & Rowntree, 1929).

The blood volume is increased. Seckel has recorded three cases of congenital heart disease with high blood volumes.

Anaemia. (Brown & Rowntree, 1929).

Hypochromic anaemia results in a low blood volume, mainly, but not solely, due to decrease in the cellular elements.

The Blood Volume in Nephritis.

Study of the literature shows that different workers have obtained very inconsistent and discordant results in the estimation of the blood and plasma volumes in nephritis.

Brown & Rowntree (1928) studied twelve adult cases of acute/

acute haemorrhagic nephritis during the oedematous phase and they found an average blood volume of 75 ml. per Kg. of corrected weight, the corrected weight being the normal weight for the patient's age and height. The range of values was from 59 to 105 ml. per Kg. corrected weight. The plasma volume averaged 52.5 ml. and ranged from 38 to 70 ml. Kg. corrected weight. These workers also studied nine adults with nephrotic nephritis during an oedematous phase and found the blood and plasma volumes considerably higher than in the patients with acute nephritis. The blood volume averaged 96.4 ml. per Kg. corrected weight with a range of 84 to 105 ml. per Kg., while the plasma volume averaged 59.4 ml. per Kg. corrected weight and ranged from 42 to 79 ml. per Kg. Brown & Rowntree summarised their results by stating that they found no evidence of a hydraemic plethora in glomerulo-nephritis with oedema nor did they recognise any uniform changes in the blood and plasma volumes during diuresis. In nephrotic nephritis they found an increased plasma volume unaffected by oedema or diuresis.

Bock (1921) estimated the blood and plasma volumes in four patients with chronic nephritis and in one of them estimations were made during oedema and after it had passed off. He considered that the plasma volume changes little, or not at all, in oedema, but appears to deduce this from the fact that in his experiments, the plasma volume retained the same/

same relationship to weight during and after oedema. He did not make any allowance for the unnatural increase in the patient's weight due to the oedema, which of course, obscures the rise in the relative plasma volume. It is not clear from what type of nephritis his patients were suffering.

The following figures are calculated from Bock's data on one of his patients, a girl aged 16 years, and show that considerable alteration in the plasma volume did in fact take place.

Date.	Wt. in Kg.	Oedema.	Plas. Vol. % of body wt.	Total Plasma Vol.ml.	Total Blood Vol.ml.	Hb. %	R.B.C. mils. per c.mm.
Jan.9	54.5	+++	4.0	2200	2587	45	2.0
Jan.24	46	++	4.1	1900	2317	61	2.5
Feb.3	41	0	4.1	1690	1965	61.5	2.4

Harris & Gibson (1939) also found the plasma volume increased in nephrotic nephritis and considered that there is no consistent change in the plasma volume with variations in oedema although the results which they published show in most of their cases, a marked fall of plasma volume during oliguria and oedema and a rise in volume with diuresis and absence of oedema. Many of their patients were anaemic and so presumably had a super-added infection, and in some patients, the successive investigations were several months apart and are therefore not readily comparable with one another. These workers/

workers concluded that the volume of the plasma is unrelated to the stage or the type of nephritis and is dependent only on the inter-relationship of serum albumin, non-protein nitrogen and degree of anaemia.

Linder et al.,(1924) investigated the plasma volume in four patients with nephrotic nephritis with the special object of finding out whether the low plasma protein was the result of dilution of the blood. They concluded from their results that the plasma volume was either uninfluenced by the presence of oedema or else that it was actually diminished when anasarca was extreme and that in nephrotic nephritis hydraemic plethora does not occur.

Darrow (1926) studied three children with nephrotic nephritis and oedema and all had a low blood and plasma volume during oedema which rose to normal after oedema had subsided. He noted that the decrease in the serum proteins is therefore greater than the apparent decrease because the plasma volume is also reduced considerably.

Waterfield (1931) used the carbon monoxide method to examine five patients with 'renal oedema' and found the plasma volume was abnormally low during increasing oedema and rose as oedema disappeared. However, many complicating factors were present in his patients; one had 'severe heart failure'; another had completed a pregnancy only four weeks previously, and in a third the reduction of oedema was obtained by multiple/

multiple skin punctures.

It has been shown that the diuresis induced by Salyrgan is accompanied by a diminution in plasma volume (Evans & Gibson,1937). It is suggested that the failure to obtain consistent results in these investigations is due to one or more of the following causes.

1. Insufficient differentiation of the type of oedema; for example, into nephritic, nephrotic and cardiac.
2. Failure to time the estimations to coincide with rapid increase or rapid decrease in oedema, and to differentiate the two groups in appraising and presenting the results. For example, a patient whose normal weight is 30 Kg. and whose weight when oedema is maximal is 40 Kg. may have a blood volume estimation done when he weighs 35 Kg. and has well marked oedema. Such an estimation remains valueless unless it is known whether it was obtained while oedema was increasing to 40 Kg. and the disease was active, or while oedema was diminishing and recovery in progress. In none of the published accounts is this essential information provided.
3. An insufficient number of plasma volume estimations in some of the series.

4./

4. The presence of complicating diseases or circumstances and the use of artificial measures, such as multiple skin puncture, to obtain reduction of oedema.

The technique adopted and some preliminary experiments.

No prolonged fasting is necessary but the estimation should not be made within three hours of the last meal. As a rule the patient had a light breakfast at 7 a.m. and the estimation was done about noon.

The solution for injection was prepared immediately before it was required by adding 10 ml. of trebly distilled water to 0.1 gm. of sterile congo red (B.D.H. Ltd.) and by warming the mixture to procure complete solution. The patient's height and weight were measured, the blood pressure taken, and a red cell count and haemoglobin estimation was done on capillary blood from the ear. After cleaning the skin a fine Wasserman needle was inserted into a vein at the left elbow and 7 or 8 ml. of blood was withdrawn and added to 2 ml. of 1.1% potassium oxalate in a 15 ml. graduated centrifuge tube by detaching the syringe from the needle and leaving the needle in the vein. A long narrow syringe designed for accurate measurement and containing exactly 4 ml. of 1% congo red was attached to the needle and the dye rapidly injected and the needle withdrawn. Exactly 4 minutes after completing/

completing the dye injection a needle was inserted to a vein at the right elbow and about 8 ml. of blood withdrawn and added to 2 ml. 1.1% potassium oxalate in a 15 ml. graduated centrifuge tube. Both blood samples were covered with a thin layer of liquid paraffin to prevent evaporation and centrifugalised at 2500 revs/min. for at least 30 minutes. After the centrifugalisation, the volume of the oxalated plasma and of the packed red cells was noted in each sample and the haematocrit and plasma dilution factor calculated. The clear plasma and the dyed plasma were pipetted off the red cells and a standard solution of 1/400 congo red was made up using 1 ml. of the 1% solution made up for injection, and distilled water. The dyed plasma and water were then compared with the undyed plasma and the standard solution in a Burker compensating colorimeter. The mechanism of the colorimeter is such that the standard is set at '10' and the relationship of the unknown is obtained from a vernier scale after matching. Five separate readings were made from the colorimeter and the average of the last four was taken as the true reading. The circulating plasma volume was then calculated from the following formula.

$$\text{Plasma volume} = \frac{N\ D\ C\ U}{S} + \text{ml. plasma in 2nd sample.}$$

where D = dilution of the standard i.e. 400
N = ml. of 1. congo red injected i.e. 4
C = correction for dilution of plasma by oxalate solution i.e. $\frac{\text{ml. of diluted plasma} - 2}{\text{ml. of diluted plasma}}$

U/

U = reading of unknown on colorimeter
S = reading of standard on colorimeter i.e. 10

The total blood volume was then calculated from the plasma volume by reference to the haematocrit.

$$\text{Blood Volume} = \frac{\text{Plasma volume x 100}}{\text{Plasma volume \% (haematocrit)}}$$

The red cell volume is the difference between the blood volume and the plasma volume. For purposes of comparison the results were then expressed as ml. per Kg. of body weight, ml. per Sq. M. body surface; and fraction of body weight. The surface area was obtained by reference to nomograms based on Dubois (1927) formula.

$$\text{Log } A = \text{Log } W \times 0.425 + \text{Log } H \times 0.725 + 1.8564 \quad (c)$$

where A = surface area in Sq. metres.
W = body weight in Kilograms.
H = height in centimetres.
C = a constant obtained by experiment (Dubois 1927).

The formula has a maximum error of 7% in children. In estimating the blood and plasma volumes as a fraction of the body weight they must be converted from volumes to weights and for this purpose a Specific Gravity of 1.030 for plasma and 1.060 for blood was adopted as sufficiently accurate.

In all patients with oedema, the relationship between blood or plasma volume and weight has been calculated on the basis of the "oedema-free weight", that is to say, the lowest weight to which the child fell subsequently, when oedema had passed off and normal water balance was restored.

A/

A typical experiment is recorded in Appendix 3.

The congo red used in these experiments was stated to be non-toxic by the manufacturers (Messrs. B.D.H. Ltd.,) but it was decided to conduct experiments on rabbits, before testing the method on human beings, in order to prove the non-toxic nature of the dye and to practice the technique. Slight modifications of the technique outlined above were required in these tests. The standard used was a 1 - 300 solution of congo red and only 3 or 4 ml. of blood were obtained as blood samples. It was found that rabbits blood required a stronger concentration of oxalate than human blood, partly because of its greater fragility and partly because the samples were obtained so slowly. The animal was fixed in a special box and the marginal vein of the left ear cut, after shaving off the surrounding fur. After securing a specimen of blood, 0.5 ml. of 1% congo red was injected into the vein and after 4 minutes, the marginal vein of the right ear was cut and a second sample of blood obtained. It took 3 to 5 minutes to obtain these specimens.

Four experiments were done. One of these was a pure toxicity experiment. One ml. of congo red was injected and no attempt made to estimate the blood volume. Although the concentration of the dye in the rabbit's blood was at least six times greater than it is with the usual quantity given to human beings, no evidence of toxicity was observed. The

results/

TABLE 4.

BLOOD VOLUME EXPERIMENTS ON RABBITS.

Number of Experiment.	Weight Kg.	Plasma Volume ml./Kg.	Blood Volume ml./Kg.	Blood Volume Total ml.
1	1.200	71	110	132
2	2.810	35	67	189
3	2.840	50	74	211
Average.	2.280	52	78	177

results of the other three experiments are recorded in Table 4 and when they are compared with the observations of others (Table 1) they correspond most closely with those of McQuarrie & Davis, using the same method, and the plasma volumes are similar to those of Nice & Katz, who also used Keith, Rowntree and Geraghty's method.

As a final test, the method was used on three normal healthy, male adults. In these tests, the subject rested for only 30 minutes before the estimation was made and 10 ml. of the dye were injected instead of 4 ml. In no case did the subject recognise any ill effect and all resumed their normal duties immediately after the final sample of blood had been taken. When the results (Table 5) are compared with those of other workers (Table 2) they show that the technique adopted gives results within the commonly accepted standards, although the average is rather lower than Brown & Rowntree's normal standard and would have been even lower than it is but for the high blood volume of one subject (P.M.) who was of spare build. In another similar experiment not recorded above, some of the dye was accidentally injected into the tissues outside an elbow vein. The subject had only trivial discomfort for 36 hours and a deep red colour spread over the antecubital area but faded completely within a week and left no injury. When such an accident occurs the experiment must, of course, be abandoned./

TABLE 5.

THE BLOOD VOLUME IN NORMAL, HEALTHY ADULTS.

Name.	Age. Yrs.	Ht. cms.	Wt. Kgs.	S.A. Sq.M.	Haematocrit R.B.C. Vol. %.	PLASMA.			RED CELLS.			WHOLE BLOOD.		
						ml./Kilo	ml./Sq.M.	Total ml.	ml./Kilo	ml./Sq.M.	Total ml.	ml./Kilo	ml./Sq.M.	Total
T.C.	25	181	72.2	1.90	45.9	41	1551	2946	35	1316	2500	75	2866	5446
A.R.	25	174	63.8	1.76	47.9	40	1459	2567	37	1885	2359	77	2799	4926
P.M.	25	186	69.6	1.92	48.2	51	1806	3567	48	1729	3312	99	3592	6886
Average	25	180	68.5	1.86	47.3	44	1605	3027	40	1643	2726	84	3086	5753

abandoned.

In view of the satisfactory nature of these preliminary trials, it was decided to proceed with the method in the study of sick children.

OBSERVATIONS ON THE BLOOD AND PLASMA VOLUME IN NEPHRITIS.

Fifty eight plasma volume estimations have been done on 28 patients with various types of nephritis. The distribution of these tests is shown in Table 6 and great importance is attached to the differentiation of the types of the disease and, in acute haemorrhagic nephritis and in nephrotic nephritis, to the stage of oedema at which the estimation was made.

Details of each of the estimations is recorded in appendix 4.

Acute Haemorrhagic Nephritis.

Twenty five plasma volume estimations were done on twelve patients with acute nephritis. The results have been separated into three groups for purposes of comparison, namely those done,

1. When the patient was markedly oedematous in the initial stage of the disease and before diuresis had become fully established.
2. When the oedema was subsiding and there was well marked diuresis.
3. When all oedema had disappeared, diuresis had passed off, and the patient was convalescent.

The/

TABLE 6.

DISTRIBUTION OF PLASMA VOLUME ESTIMATIONS IN NEPHRITIS.

Acute Haemorrhagic Nephritis.	Acute stage with oedema	9
	Stage of diminishing oedema	9
	Oedema-free stage	7
Nephrotic Nephritis.	State of increasing oedema	8
	State of diminishing oedema	4
	Oedema-free stage	5
Chronic Haemorrhagic Nephritis		11
Nephrosclerosis		5
Total Experiments		58

The results have been expressed in millilitres per Kilogram of body weight as well as total volume because this provided a useful means of comparing the extent of the change in volume in children of different age and weight. But, as was already explained, a child's weight is greatly augmented by the mass of oedema and it would be erroneous to relate the plasma volume to the artificially high weight of a grossly oedematous child. For this reason, in each case the "oedema-free weight", that is the lowest weight which the child reached after all oedema had disappeared, was used for calculating the plasma volume per Kilogram. For instance, the child A.D. (Table 7 and Appendix 4) weighed 29.6 Kg. on the day of her first plasma volume estimation, 25.1 Kg. on the day of the second, and her final oedema-free weight was 23.6 Kg. but the volumes of 61, 44, 68 ml./Kg. in table 7 were each calculated on a weight of 23.6 Kg.

This seems the most useful and reasonable way of arriving at a basis for comparison although it is realised that the final weight, coming as it does after an acute illness with dietetic restrictions, must usually be below the child's normal weight in health. This is the reason why the plasma volume is high in relation to body weight in nearly all patients in this group. Taking this into account, the average results (table 7) show that the plasma volume is not above normal while the child has gross oedema; that it becomes/

TABLE 7.

PLASMA VOLUME IN ACUTE NEPHRITIS.

NAME. AGE.	Stationery oedema Before Diuresis		Subsiding oedema During Diuresis		No oedema after Diuresis	
	Total ml.	ml./Kg.	Total ml.	ml./Kg.	Total ml.	ml./Kg.
G.C. 7 yrs.	865	49			901	51
F.C. 6 yrs.	986	65	645	43		
R.McP. 4 yrs.					717	53
A.J. 5 yrs.	1083	67			925	57
A.D. 10 yrs.	1433	61	1039	44	1613	68
R.J. 10 yrs.					1474	58
E.G. 8 yrs.	1067	57	972	51	1385	73
R.D. 10 yrs.	1573	68	1190	52		
E.G. 10 yrs.	1151	59	978	50		
J.M. 5 yrs.	882	61	763	53	1088	76
S.D. 4 yrs.	869	59	644	44		
G.H. 8 yrs.			1024	52		
/*			996	51		
Averages.		61		49		62

becomes considerably reduced when the kidney recovers its function and diuresis occurs, and that it returns to normal when the diuresis comes to an end and the fluid balance is restored.

The difficulty of considering the changes in the total volume of red cells as a group is manifest by study of the individual results in Table 8 and by reference to the haemoglobin levels in appendix 4. Some patients were anaemic when first examined (G.C.) and recovered during treatment, while others developed anaemia during their illness (A.D.; F.C.; J.M.) and recovered during convalescence.

It is to be expected therefore, that the volume of red cells will be higher after illness than it was during the course of the disease.

When the average figures in Table 8 are compared with those in Table 7, they show conclusively that the fluctuation observed in the red cell count associated with diuresis and diminishing oedema and described in Part 2 of this thesis is due to changes in the plasma volume and not to anaemia. The average red cell volume during the initial stage was 37 ml./Kg. and the plasma volume was 61 ml./Kg. and during the period of subsiding oedema and diuresis the red cell volume remained at 37 ml./Kg. while the plasma volume fell to 49 ml./Kg. and the rise in the cell count observed at this stage is seen to be due to loss of fluid

from/

TABLE 8.

RED CELL VOLUME IN ACUTE NEPHRITIS.

NAME. AGE.	Stationery oedema Before Diuresis		Subsiding oedema During Diuresis		No oedema After Diuresis	
	Total ml.	ml./Kg.	Total ml.	ml./Kg.	Total ml.	ml./Kg.
G.C. 7 yrs.	461	26			710	40
F.C. 6 yrs.	622	41	488	32		
R.McP. 4 yrs.					409	30
A.J. 5 yrs.	623	38			508	31
A.D. 10 yrs.	916	39	702	30	1158	49
R.J. 10 yrs.					832	32
E.G. 8 yrs.	764	40	787	42	858	46
R.D. 10 yrs.	761	33	793	34		
E.G. 10 yrs.	690	36	734	38		
J.M. 5 yrs.	595	44	642	47	919	68
S.D. 4 yrs.	497	34	504	34		
G.H. 8 yrs			782	40		
"			654	33		
Averages		37		37		42

from the blood stream. During the subsequent convalescence the red cell volume rose to 42 ml./Kg. indicating recovery from a mild anaemia, while the plasma volume returned to its normal level of 62 ml./Kg.; therefore during convalescence there was an increase in the total number of circulating red cells although the red cell count was falling because the plasma was increasing even more rapidly.

These results also demonstrate that some children with acute haemorrhagic nephritis develop a moderate degree of genuine anaemia during the course of their illness and the extent of this anaemia, obscured as it is by variations in the plasma volume, can be recognised only by estimation of the total circulating red cell volume.

Nephrotic Nephritis.

Seventeen plasma volume estimations were done on five patients with nephrotic nephritis. Since this is not an acute disease, and is not usually accompanied by any wasting, the oedema-free weight of these children is about the expected weight for their age and consequently, the plasma volume related to weight (Table 9) is quite normal during the quiescent phase of the disease. The average of the results does not demonstrate any very significant change in the plasma volume during the stage of increasing oedema and oliguria, but when oedema is diminishing and diuresis has commenced there is a very marked increase

in the plasma volume. The fact that when urinary excretion is low there is no increase in plasma volume and, that it is considerably increased during the period of diuresis, strongly suggests that in nephrotic nephritis it is the change in plasma volume which determines the volume of urinary excretion. It is very difficult to recognise when these children are developing a large accumulation of oedema, since during the active phase of the disease their weight and urinary excretion fluctuate considerably every few days and it is considered probable that if the plasma volume estimations in the first column of Table 9 could be timed more accurately to synchronise with a period of rapidly increasing oedema, the volumes disclosed would be lower than those recorded. This is supported by the results obtained in the patient T.M. in whom the estimations were well timed to coincide with rapid fluctuations of oedema.

The red cell volume of these children (Table 10) is considerably above normal and gives a total blood volume above normal and sometimes very greatly above normal. These patients are very liable to develop a sudden anaemia of considerable degree as a result of an acute infection and the very wide fluctuation in their red cell volumes is attributed to the fact that they were often very anaemic or were recovering from anaemia. The combination of wide fluctuations in the plasma volume in association with changes in oedema and wide fluctuations in the red cell volume associated with infection/

TABLE 10.

VOLUME OF CIRCULATING RED CELLS IN NEPHROTIC NEPHRITIS.

NAME. AGE.	Increasing Oedema Oliguria		Diminishing Oedema Polyuria		No Oedema	
	Total ml.	ml./Kg.	Total ml.	ml./Kg.	Total ml.	ml./Kg.
J.McD.	1127	61	1214	65	861	46
5 yrs.	1175	63	960	52		
T.M.	1043	59	729	41	801	45
7 yrs.	841	47				
	422	24				
I.D. 5 yrs.	288	24				
W.G. 7 yrs.			1434	77	860	46
C.McG.	680	49			557	40
3 yrs.	668	48			708	51
Averages		47		59		46

infection produces a combination of changes in the blood counts and haemoglobin estimations which may be difficult to interpret without these absolute measurements. No explanation can be offered for the surprisingly high red cell volume in these patients.

Nephrosclerosis.

A blood volume estimation has been made on each of five children with nephrosclerosis. They were typically thin, undernourished children and all had been on restricted diet for variable periods. This explains their high blood and plasma volumes in relation to weight (Table 11) and is comparable to the findings in healthy subjects who are of spare build. If these children had been of normal weight for their age and height, the relative blood volumes would have been perfectly normal. The results show that the patients who were anaemic (R.L.; P.B.) as a result of haemorrhage or infection, had a normal plasma volume and a subnormal red cell volume. Red cell counts and haemoglobin estimations in these patients provide an accurate representation of the degree and course of the anaemia.

Chronic Haemorrhagic Nephritis.

Eleven plasma volume estimations on six cases of chronic haemorrhagic nephritis reveal the high relative plasma/

TABLE 11.

BLOOD AND PLASMA VOLUME IN NEPHROSCLEROSIS.

NAME. AGE.	Haemoglobin (% Haldane)	PLASMA VOLUME		BLOOD VOLUME	
		ml./Kg.	Total ml.	ml./Kg.	Total ml.
J.C. 6 yrs.	86	58	742	104	1318
M.McN. 9 yrs.	108	60	1001	109	1810
J.D. 9 yrs.	74	62	986	101	1611
R.L. 10 yrs.	43	55	988	71	1263
P.B. 7 yrs.	58	52	724	79	1104
AVERAGES.	74	57	888	93	1421

plasma volume expected in thin, underweight children with anaemia (Table 12). The mean height of these children was 125 cms. and their mean weight was 21.2 Kg. Had they been normal healthy children with an average height of 125 cms. their average weight would have been 26 Kg. (Holt & McIntosh) and from this figure using Brown & Rowntree's standards, their normal plasma and red cell volumes can be calculated.

Height cms.	Weight Kg.	R.B.C. Vol. ml.	Plasma Vol. ml.	Blood Vol. ml.
125	26	910	1378	2288

The actual results obtained in these children were:-

Height cms.	Weight Kg.	R.B.C. Vol. ml.	Plasma Vol. ml.	Blood Vol. ml.
125	21.2	747	1301	2048

This shows that they have an average deficiency of 77 ml. of plasma and 163 ml. of red blood cells or expressed as a percentage their plasma volume is 5.7% below normal and their red cell volume is 18% below normal. It is obvious that the reduction in the red cell counts and haemoglobin estimations in this group of children (see Part 111) is due to a genuine anaemia and not to dilution of the blood by an excess of plasma.

Discussion.

In the absence of a reliable and sufficiently comprehensive standard of blood and plasma volume in normal children, it is not possible to make dogmatic statements about the changes in total blood volume which are found in the/

TABLE 12.

BLOOD AND PLASMA VOLUME IN CHRONIC HAEMORRHAGIC NEPHRITIS.

NAME. AGE.	Haemoglobin (% Haldane)	PLASMA VOLUME		BLOOD VOLUME	
		ml./Kg.	Total ml.	ml./Kg.	Total ml.
A.A. 8 yrs.	64	45	1019	66	1494
J.C. 5 yrs.	44	79	1302	105	1736
J.R. 7 yrs.	72	65	1823	106	2981
"	80	59	1662	101	2847
"	75	65	1831	108	3033
P.C. 7 yrs.	70	68	1230	107	1934
"	76	74	1334	118	2118
M.R. 6 yrs.	58	64	887	90	1245
"	60	70	964	100	1383
"	62	64	889	99	1372
A.McA. 12 yrs.	85	48	1373	84	2384
AVERAGES.	68	64	1301	99	2048

the different types of non-oedematous nephritis. The following deductions are therefore based on the assumption that the relative blood volume of children is comparable to the accepted standards in adults or else is slightly higher.

In acute nephritis, chronic haemorrhagic nephritis and nephrosclerosis (Tables 7, 8, 11, 12) the malnutrition resulting from dietetic restrictions and the influence of the disease leads to a plasma volume high in relation to body weight in contrast to the normal plasma volume in the non-oedematous phase of nephrotic nephritis (Tables 9, 10) a disease in which general nutrition is not seriously affected. It should be noted that the blood volume in these first three diseases is raised only relative to weight and that the disease leads to no change in the total blood volume except in association with oedema or anaemia. Oedema causes changes in the plasma volume and anaemia causes changes in the red cell volume.

The results obtained by the plasma volume estimations confirm the hypothesis made from the previously observed variations in the red cell count in the course of acute nephritis and nephrotic nephritis, that the fluctuations in the red cell counts are due to variations in the plasma volume and that the mechanism of recovery in these two diseases is entirely different. An attempt has been made to show these differences diagramatically in the accompanying/

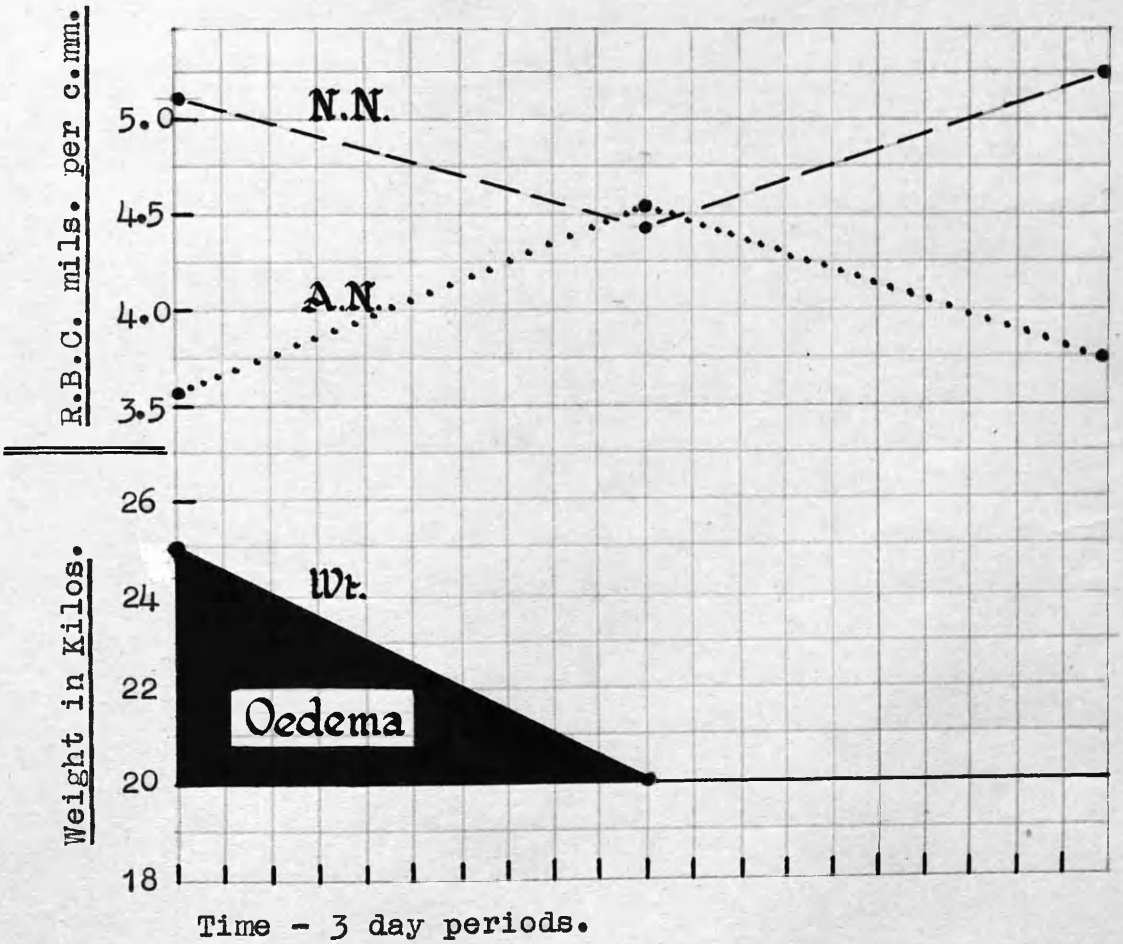
accompanying series of charts which deal with the red cell counts, plasma volumes, oedema and diuresis of children with acute nephritis and nephrotic nephritis. A detailed consideration of the cell counts has already been made in Part 11.

Chart 1. represents the relationship between the red cell counts and the stage of oedema and chart 2. illustrates the relationship between the same counts and the volume of urinary excretion. Charts 3 and 4 deal with the children in whom blood volume estimations were made. The red cell counts in these children were similar to those in the larger series illustrated in charts 1 and 2 and the cell counts are compared in charts 3 and 4 with the changes in plasma volume and the volume of urinary excretion. It is evident from these charts that the changes in the red cell count can be attributed to variations in the plasma volume.

In acute nephritis (chart 3) with oedema and before diuresis had commenced, the average plasma volume was 61 ml. per Kg. but during diuresis with rapidly diminishing oedema the plasma volumes were the lowest recorded in the whole series, averaging only 49 ml. per Kg. and then some time later, when the water balance of the body had been re-established, the plasma volume had returned to approximately the first level at 62 ml. per Kg. Patients with acute nephritis do not as a rule arrive in hospital during the phase when oedema is increasing and so it has not been possible/

CHART 1.

RED CELL COUNTS DURING AND AFTER OEDEMA.



Body weight in Kilos.

Red cell count in acute nephritis.

Red cell count in nephrotic nephritis.

CHART 2.

RED CELL COUNTS DURING DIURESIS.

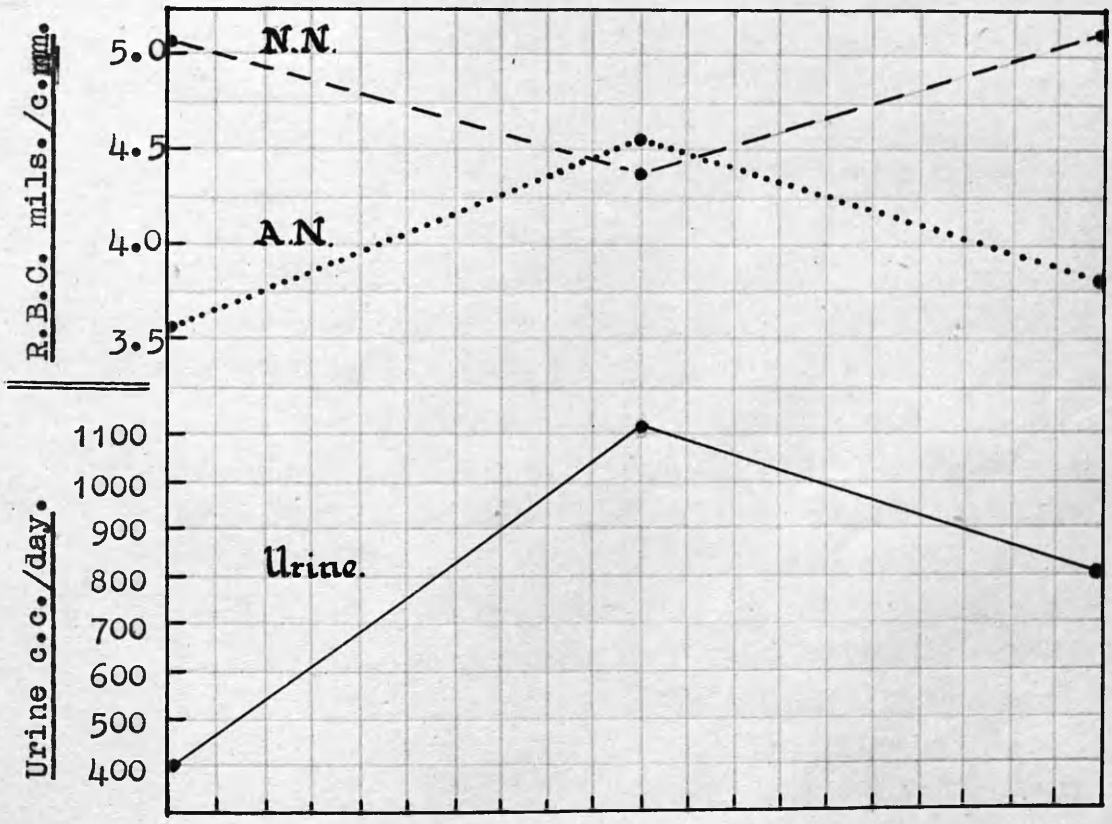
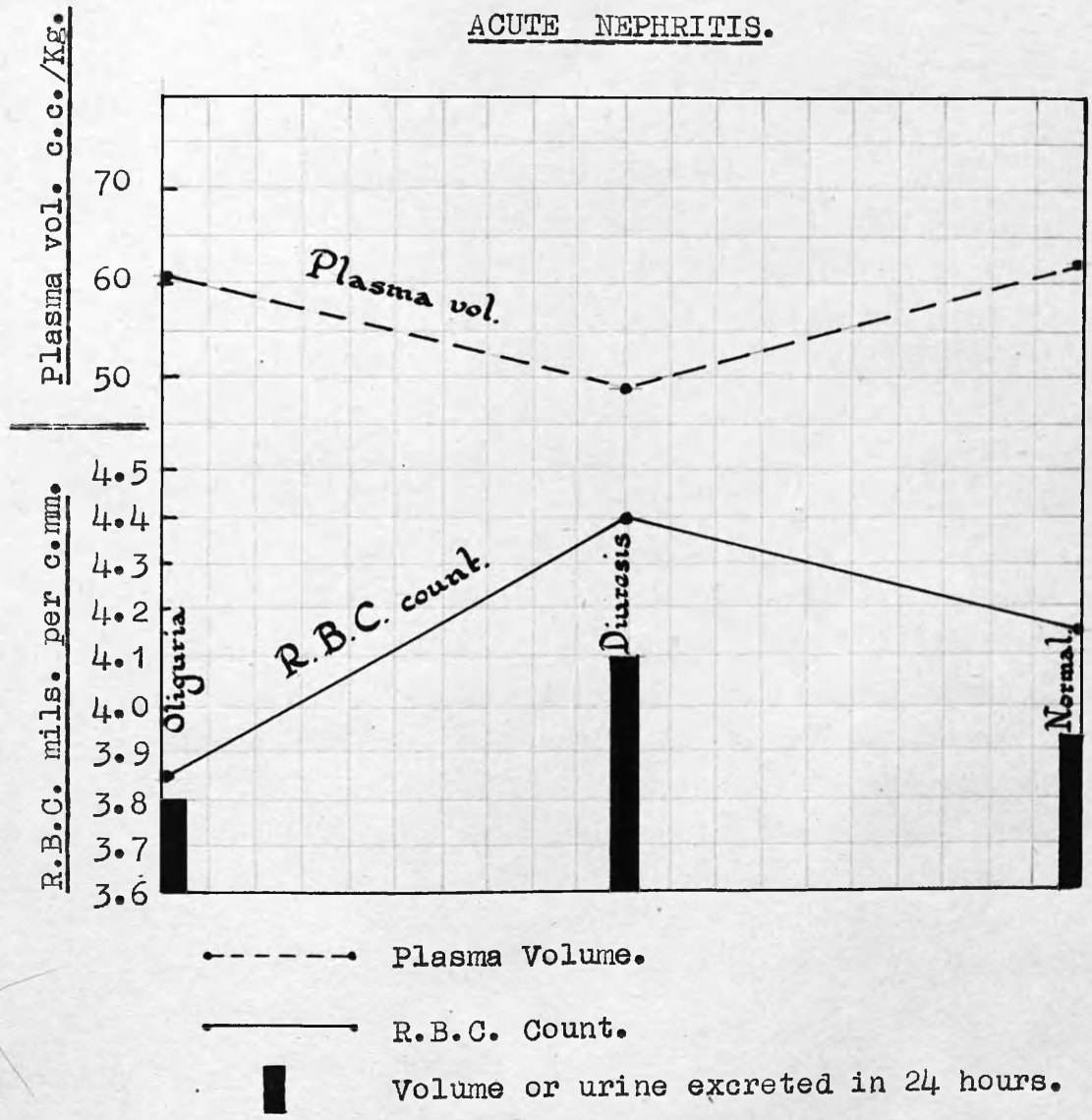


CHART 3.

ACUTE NEPHRITIS.



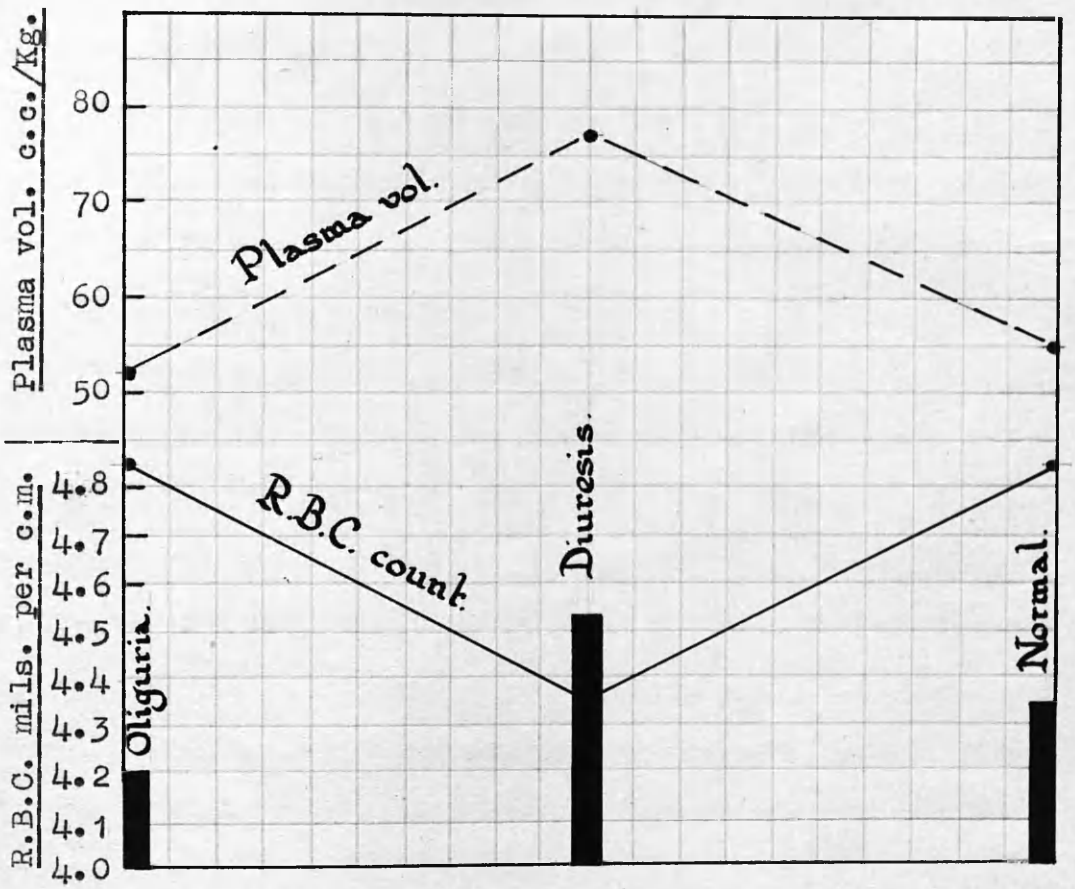
Plasma Volume.

R.B.C. Count.

Volume or urine excreted in 24 hours.

CHART 4.

NEPHROTIC NEPHRITIS.



Plasma Volume.

R.B.C. count.

Volume of urine excreted in 24 hours.

possible to make any estimations at this stage, but since this is the time when the kidney function is most seriously affected, it is considered probable that the plasma volume is then increased.

It is suggested that in the initial stage of acute nephritis, when water excretion by the kidneys is impaired, fluid is dammed back and the plasma volume rises, the excess water then passes out from the blood stream into the tissue spaces, producing oedema and as it does so the plasma volume gradually returns to normal. When the kidneys recover their function, fluid is drawn from the blood to eliminate accumulated waste products, the plasma volume is reduced below normal and the fluid gradually returns from the tissue spaces to the blood and oedema diminishes. When accumulated waste products have been eliminated and the excess of fluid in the tissues has disappeared, diuresis ceases and the plasma volume returns to normal.

In nephrotic nephritis (chart 4) the changes in the blood volume are quite different. In this case it was possible to time some of the plasma volume estimations so that they were done during a period when the oedema was still increasing and oliguria was more or less marked. At this stage, the plasma volumes averaged 52.5 ml. per Kg. During the second phase, that of diminishing oedema and diuresis, the highest plasma volumes of the whole series were obtained and averaged

77.5 ml. per Kg. in complete contrast to the lowest volumes of the series obtained during this phase of diminishing oedema in acute nephritis. During the third stage, when oedema had disappeared and diuresis had ceased, the normal value of 55 ml. per Kg. was obtained. It is suggested that these results provide good evidence that the oedema of this disease is not due to failure of renal function. Initially, fluid passes from the blood into the tissue spaces and oedema, a low plasma volume, and oliguria result. During recovery, fluid pours back into the blood causing subsidence of oedema and a high plasma volume, and at the same time the kidneys excrete the excess of water in the blood with resultant diuresis. Finally, when fluid ceases to pass from the tissues to the blood in excessive quantity, the plasma volume returns to the normal level and diuresis ceases.

In acute nephritis, alteration in the volume of fluid excreted by the kidneys is the cause of the changes in the plasma volume, but in nephrotic nephritis it is the change in the plasma volume that determines the volume of renal excretion.

As no previous investigator has differentiated the phases of the different types of nephritis in presenting the results of blood volume estimations it is not possible to make a close comparison of the results obtained in this study with those of other workers.

The/

The observations in nephrotic nephritis are, however, in general agreement with those of Brown & Rowntree (1928) in that very definitely high blood and plasma volumes have been found, particularly during the stage of diminishing oedema.

Bennhold (1926) drew attention to the fact that congo red disappears more quickly from the circulation in patients with nephrotic nephritis than in normal persons and this was suggested as a reason for the high blood volume found. Brown & Rowntree (1928) estimated the rate of excretion of congo red from the blood and claimed that it was only 1.5% faster than in normal subjects and they stated that the amount of dye lost during the four minute period before the blood sample was taken was not sufficient to alter the result significantly.

It is of great interest to compare the changes in plasma volume which have been found to occur in nephrotic nephritis, with the plasma volume studies of Walters et al., (1947) in ex-prisoners of war who were suffering from severe malnutrition, hypoproteinaemia, and oedema. In both groups of patients, the serum albumin level was greatly reduced and there was widespread oedema. The fluctuations in the plasma volume associated with recovery were identical in the two groups. While oedema was extreme the plasma volume was about normal or less than normal, as oedema diminished, the plasma volume rose far above normal and some weeks later, when/

when oedema had disappeared and diuresis ceased, the plasma volume returned to a normal level.

Walters draws attention to the fact that the increase in serum albumin which occurs as recovery takes place, is much greater than the rise in serum protein concentration suggests, because the plasma volume is also greatly increased.

The blood volume tests have confirmed the observation, made from other haematological data, that in severe cases of acute haemorrhagic nephritis, a mild and temporary anaemia may develop and that in chronic haemorrhagic nephritis there is a more severe and persistent anaemia which cannot be attributed to hydraemia.

GENERAL SUMMARY.

As a preliminary to the study of anaemia in nephritis, investigations were made upon the effect of oedema on the accuracy of red cell counts done on capillary blood and it has been shown that the presence of oedema leads to a dilution of the capillary blood flowing from a puncture in the lobe of the ear. There is a false lowering of the red cell count by about 2.5 per cent but in view of the technical inconvenience and increased experimental error entailed in obtaining specimens of venous blood for cell counts it was decided to use capillary blood as a routine procedure while bearing in mind the inevitable small error which arises in oedematous individuals.

Serial red cell counts done throught the course of acute haemorrhagic nephritis and nephrotic nephritis have shown that the concentration of red cells in the blood varies with the degree of oedema and the volume of urinary secretion and direct plasma volume estimations have proved that these changes in the red cell count are due to fluctuations in the plasma volume. In acute haemorrhagic nephritis as recovery occurs and diuresis begins, there is a rise in the red cell count which reaches a maximum of

about/

about 700,000 red cells per c.mm. of blood at the height of diuresis and thereafter gradually returns to its previous level with the restoration of normal water balance and kidney function during convalescence.

In nephrotic nephritis, however, the red cell count rises with increasing oedema and oliguria and falls sharply during diminishing oedema and diuresis.

As an explanation of these findings it was shown that during the active stage of acute nephritis when renal function is impaired, fluid is damned back and accumulates in the blood whence it is pushed out into the tissues. During the stage of recovery, when the kidney resumes its function, fluid is withdrawn from the blood stream to excrete accumulated waste products and the plasma volume falls.

In nephrotic nephritis on the other hand, the kidneys seem to play a passive role. During the active stage of the disease when oedema is increasing, fluid escapes from the blood into the tissues and the plasma volume decreases and oliguria results. When recovery begins and oedema diminishes, fluid returns to the blood stream, the plasma volume is increased and the excess fluid in the blood is excreted by the kidneys with resultant diuresis.

As the total red cell volume of the blood remains about constant, it can readily be understood how these fluctuations in plasma volume can produce changes in the red cell count which suggest an increasing anaemia but are in reality due to a diminishing haemoconcentration.

The contradictory results obtained by workers studying anaemia in nephritis has been discussed and some explanations for the inconsistencies have been proposed. The necessity to consider different types of kidney disease independently has been emphasised and it has been shown that the typical chronic, intractable anaemia of nephritis occurs only in the chronic haemorrhagic type of the disease. This anaemia is orthochromic and normocytic, is accompanied by a slight leucocytosis, and there is a normal proportion of reticulocytes among the red cells. It is moderately severe in degree and large doses of iron, liver extract, and vitamin C failed to produce any amelioration of the anaemia.

A number of investigations designed to show the type and etiology of the anaemia have been described and it was concluded that while chronic haemorrhage may, per se, rarely lead to some degree of anaemia, in the vast majority of cases the anaemia is due to some factor detrimental to the development of the red cell. Gastric hypochlorhydria/

hypochlorhydria is a frequent accompaniment of anaemia in nephritis but is nor invariably present nor is the severity of the hypochlorhydria proportionate to the degree of anaemia.

The methods of estimating the blood volume have been discussed and the influence of a number of factors upon the red cell volume and the plasma volume has been described. The inadequacy of published records of the blood volume in childhood was noted but it was shown that it is safe to assume a child has a blood volume relatively larger than an adult. Review of the published work on the changes which occur in the blood volume during nephritis showed a remarkable failure to obtain consistent results and certain reasons for this inconsistency were suggested. The blood volume experiments described in the present study showed that:

1. Oedema is accompanied by changes in the plasma volume.

2. An acute pyogenic infection occurring in a patient with nephrotic nephritis leads to a fall in the total volume of circulating red cells.

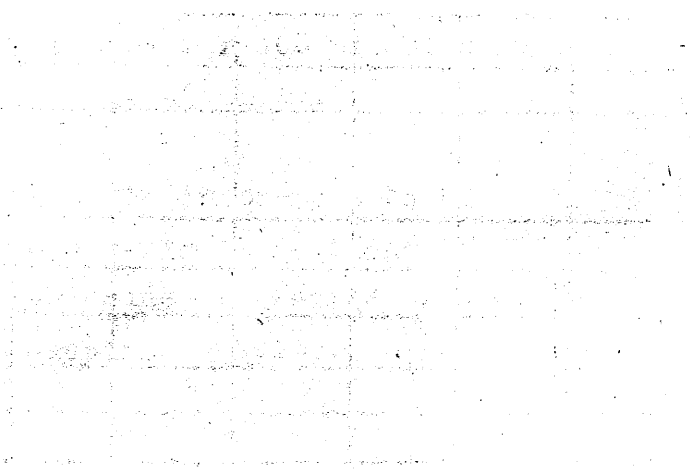
3. An acute infection or a haemorrhage in a patient with nephrosclerosis leads to a fall in the total red cell volume.

4. Chronic haemorrhagic nephritis is accompanied by a moderately severe reduction in the total red cell volume.

All/

All of these changes are shown clinically by a fall in the haemoglobin level and in the red cell count but not all of them indicate the presence of anaemia. In the first group there is no anaemia; in the second and third groups there is a temporary anaemia with active haemopoiesis, and if the infection or haemorrhage is controlled spontaneous recovery from the anaemia occurs rapidly.

In the fourth group there is an orthochromic, normocytic anaemia which is moderately severe and quite intractable, and provides as good a guide to the stage, course and pronosis of the disease as any other single factor.



APPENDIX ONE

EXPLANATION OF PROFORMA.

Name.....

Date.	
Blood.	
R.B.C.(e)	Red blood cells in capillary blood (millions per c.mm.)
R.B.C.(v)	Red blood cells in venous blood (millions per c.mm.)
W.B.C.(e)	White blood cells per c.mm. of capillary blood.
W.B.C.(v)	White blood cells per c.mm. of venous blood.
Hb. (e)	Haemoglobin of capillary blood (per cent, Haldane) (100% - 13.8 gms.)
Hb. (v)	Haemoglobin of venous blood (per cent, Haldane.)
C.I.(e)	Colour index - capillary blood.
C.I.(v)	Colour index - venous blood.
Reticulocytes.	Per cent of red cells.
Film.	
Blood Pressure.	Millimetres of mercury. Systolic/Diastolic.
Weight.	Kilograms.
Oedema.	Empirical signs (Rennie 1933).
N.P.N.	Non-protein nitrogen - mgms. per 100 ml. of blood.
Blood Cl.	Whole blood chlorides in mgms. of NaCl. per 100 ml. of blood.
Plasma Cl.	Plasma chlorides in mgms. of NaCl. per 100 ml. of blood.
R.B.C. (Vol.)	Packed red cells per cent of whole blood.
Serum Protein.	Grammes per 100 ml. of serum.
Van den Berg.	Empirical units.
Urine.	
Alb.	Albumin in parts Esbach.
Blood.	Red cells in millions per 24 hours excretion.
Cl.	Chloride in grammes of NaCl. per 24 hours excretion.
Sp. Gr.	Specific Gravity.
Amount.	
Urobilin.	
Blood Volume.	Millilitres.
Test Meal.	

Name. Annie Anderson. Aged 8 years.

Date.	22 Oct.	29. Oct.	5 Nov.	12 Nov.	19 Nov.	26 Nov.	3 Dec.	10 Dec.	17 Dec.
Blood.									
R.B.C.(e)	3·625	3·530	3·210	2·980	3·385	3·205	3·270	3·030	3·530
R.B.C.(v)	3·580	3·550	3·250	2·900	3·245		3·250		3·810
W.B.C.(e)	13,600	9,600	9,500	9,500	11,800	6,800	8,600	8,300	10,900
W.B.C.(v)	12,800	7,200	8,800	10,600	10,600		9,200		9,600
Hb. (e)	68	70	64	64	63	66	64	64	66
Hb. (v)	69	68	66	62	63		64		68
C.I.(e)	0·9	1·0	1·0	1·07	1·0	1·0	1·0	1·0	0·9
C.I.(v)	0·9	0·9	1·0	1·0	1·0		1·0		0·9
Reticulocytes.	1·1	1·1							
Film.	✓	✓							
Blood Pressure.	124/48	114/48	98/50	110/68	120/46	108/64	108/62	114/84	96/54
Weight.	29·5	26·74	26.30	25·95	26·55	26·58	25·25	25·55	25·79
Oedema.	+	+	+	+	+	+	+	+	+
N.P.N.	28·7		34·7		42		44·8		22·5
Blood Cl.	505		546		521		536		526
Plasma Cl.	631		600		605		621		653
R.B.C. (Vol.)	29·0		24·0		26·0		28·0		32·0
Serum Protein.	5·25		5·26		5·24		5·18		5·89
Van den Berg.	NEG.		NEG.		NEG.		NEG.		NEG.
Urine.									
Alb.	2	2	2	1·25	5	2	1·25	1·5	1·25
Blood.	691	511	870	1516	937	610	281	1146	490
Cl.	1·5	2·5	0·64	1·1	1·9	1·3	0·4	1·3	0·8
Sp. Gr.	1014	1013	1012	1013	1016	1012	1013	1014	1015
Amount.	520	430	300	590	910	560	230+	560	500
Urobilin.	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.
Blood Volume.									
Test Meal.									

Name. Annie. Anderson. ... 8. years. Page 2.

Date.	24 Dec.	31 Dec.	4 Jan.	14 Jan.	21 Jan.	29 Jan.	4 Feb.	11. Feb.	18 Feb.
Blood.									
R.B.C.(e)	2.940	3.284	3.095	3.000	2.990	3.025	3.180	2.990	3.255
R.B.C.(v)		3.155		2.855			3.165		
W.B.C.(e)	9,500	8,400	8,600	8,300	8,900	9,500	9,800	9,400	10,900
W.B.C.(v)		8,400		8,900			9,000		
Hb. (e)	62	64	62	60	60	60	64	58	60
Hb. (v)		64		60			64		
C.I.(e)	1.05	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9
C.I.(v)		1.0		1.0			1.0		
Reticulocytes.									
Film.									
Blood Pressure.	106/62	104/60	100/60	106/48	106/46	104/48	102/54	98/54	110/66
Weight.	25.8	25.61	25.68	25.18	25.5	25.45	25.4	25.0	25.2
Oedema.	+	+	+	+	+	+	+	+	+
N.P.N.		53.2		34.6			44.2		30.5
Blood Cl.		515		534			509		536
Plasma Cl.		619		669			669		645
R.B.C. (Vol.)		30		30			33.3		29.4
Serum Protein.		5.59		5.68			5.62		5.21
Van den Berg.		Neg.		Neg.			Neg.		Neg.
Urine.									
Alb.	1.5	1.5	1.25	1.5	1.5	1.5	1.5	1.25	1.0
Blood.	436	360	299	93	440	164	202	282	282
Cl.	0.6	0.8	1.0	2.9	0.8	1.3	0.9	1.0	1.2
Sp. Gr.	1012	1012	1011	1013	1008	1011	1012	1012	1009
Amount.	450	600	535	1160	1120	820	420	1010	860
Urobilin.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Blood Volume.									
Test Meal.									

Name. Annie Anderson. Aged 8 years. Page 3.

Date.	25 Feb.	4 Mar.	11 Mar.	18 Mar.	25 Mar.	1 April	8 April	22 April	29 April
Blood.									
R.B.C.(e)	2·850	2·870	3·045	3·140	3·180	3·130	3·200	2·980	3·080
R.B.C.(v)									
W.B.C.(e)	8,500	8,400	10,400	4,600	15,800	12,200	12,500	10,300	10,000
W.B.C.(v)									
Hb. (e)	58	58	60	60	64	65	64	60	60
Hb. (v)									
C.I.(e)	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0
C.I.(v)									
Reticulocytes.			0·9	1·8	1·5	1·4	1·0		
Film.									
Blood Pressure.	108/40	108/42	104/58	104/48	104/56	98/52	108/64	104/48	102/44
Weight.	24·26	25·4	24·9	24·8	24·7	24·8	24·4	24·65	24·0
Oedema.	+	+	+	+	+	+	+	+	−
N.P.N.			24·8			44·2		31·4	
Blood Cl.			505			533		515	
Plasma Cl.			611			644		651	
R.B.C. (Vol.)			29·4			33·7		31·4	
Serum Protein.			4·49			5·68		5·18	
Van den Berg.			Neg.			Neg.		Neg.	
Urine.									
Alb.	1·0	1·0	1·0	1·45	2·0	2·0		+	+
Blood.	843	207	144	242	260	211		244	302
Cl.	0·6	2·1	1·0	1·2	1·6	0·9		0·4	2·3
Sp. Gr.	1012	1007	1009	1012	1008	1010		1012	1013
Amount.	430	1670	1030	310	840	880		660	1160
Urobilin.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.		Neg.	Neg.
Blood Volume.									
Test Meal.									

Treatment: 13th Mar. Ferrous sulphate 5 grains thrice daily.
12th Apr. Stop Ferrous sulphate.

Name..Annie.Anderson...Aged.8.years. Page 4.

BLOOD TRANSFUSION. ↓

Date.	6 MAY	13 MAY	20 MAY	24 MAY	3 JUNE	9 JUNE	16 JUNE	24 JUNE	29 JUNE
Blood.									
R.B.C.(e)	3.080	3.210	3.310	3.360	3.230	3.010	2.970	4.045	4.080
R.B.C.(v)									
W.B.C.(e)	9,500	9,400	8,200	8,300	12,200	9,200	4,800	11,400	11,200
W.B.C.(v)									
Hb. (e)	58	64	64	64	62	58	58	48	80
Hb. (v)									
C.I.(e)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
C.I.(v)									
Reticulocytes.		0.8	1.3	0.6	0.9	1.4	1.7		
Film.									
Blood Pressure.	106/40	108/42	114/60	108/66	98/46	100/44	118/48	118/80	106/40
Weight.	24.4	24.78	24.2	25.45	24.95	25.8	26.9	27.1	27.01
Oedema.	+	+	+	+	+	+	+	+	+
N.P.N.		26.6		24.4			23.9		
Blood Cl.		533		500			526		
Plasma Cl.		684		642			674		
R.B.C. (Vol.)		32.4		34.0			31.4		
Serum Protein.		5.18		4.94			4.62		
Van den Berg.		NEG.		NEG.			NEG.		
Urine.									
Alb.	2.5	1.0	1.0	2.0	1.5	1.0	2.0	2.0	
Blood.	450	204	221	420	90	82	126	202	
Cl.	3.1	1.4	1.4	1.6	2.6	1.5	1.4	2.2	
Sp. Gr.	1014	1013	1014	1010	1012	1012	1012	1011	
Amount.	1450	1200	960	1500	1130	1030	900	920	
Urobilin.	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.	
Blood Volume.				1494					
Test Meal.									

Name... John Reid. Aged 7 years.

Date.	12 June	20 June	26 June	4 July	11 July	17 July	24 July	12 Sept.	
Blood.									
R.B.C.(e)	3·925	4·565	3·890	4·090	3·940	3·945	4·290	3·930	
R.B.C.(v)									
W.B.C.(e)	6,000	5,900	4,600	6,100	5,500	5,400	11,500	4,440	
W.B.C.(v)									
Hb. (e)	42	80	44	45	44	44	46	64	
Hb. (v)									
C.I.(e)	1·0	0·9	0·9	0·9	0·9	0·9	0·9	0·9	
C.I.(v)									
Reticulocytes.	0·4								
Film.	✓								
Blood Pressure.	124/48	110/66	120/68	98/66	98/50	114/60	94/40		
Weight.	35·68	30·12	32·52	30·32	28·42	28·08	29·04		
Oedema.	+ +	+	+	+	∓	−	−		
N.P.N.	44·8	30·1		29·2					
Blood Cl.	444	382		441					
Plasma Cl.	−	−		−					
R.B.C. (Vol.)	39.0	41.4		40·8					
Serum Protein.	4·2	6·81		6·65					
Van den Berg.	Neg.	Neg.		Neg.					
Urine.									
Alb.	0·5	0·5	0·5	tr.	tr.	tr.	tr.	tr.	
Blood.	139	162	40	114	11	143	?	?	
Cl.	2·2	1·0	3·2	5·2	?	4·2	6·0		
Sp. Gr.	1023	1014	1005	1020	1012	1015	1016		
Amount.	410	450	1350	600	1120	450	480		
Urobilin.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.		
Blood Volume.	2981	2847		3033					
Test Meal.						✓			

Treatment: 24.7.39. Ferrous Sulphate 3 grains thrice daily.

Name... Jack Campbell... Aged 5 years.

Date.	19 July	26 July	2 Aug.	9 Aug.	17 Aug.	23 Aug.	30 Aug.		
Blood.									
R.B.C.(e)	2.440	2.730	2.565	2.775	3.065	3.295	2.990		
R.B.C.(v)									
W.B.C.(e)	6,400	10,300	13,900	10,500	13,400	11,800	12,200		
W.B.C.(v)									
Hb. (e)	44	50	50	56	54	54	52		
Hb. (v)									
C.I.(e)	0.9	0.9	1.0	1.0	0.9	0.8	0.9		
C.I.(v)									
Reticulocytes.	0.6					0.5			
Film.	✓								
Blood Pressure.	100/40	108/82	94/64	106/80	100/40	108/68	108/40		
Weight.	17.4	17.0	16.46	16.7	16.64	16.3	16.02		
Oedema.	∓	-	-	-	-	-	-		
N.P.N.	57.5		44.2		48.1		45.4		
Blood Cl.	531		457		500		552		
Plasma Cl.	647		632		605		618		
R.B.C. (Vol.)	25.4		26.7		27.6		29.8		
Serum Protein.	5.09	6.57	6.02		6.53				
Van den Berg.	Neg.		Neg.		Neg.		Neg.		
Urine.									
Alb.	4.5	3	1	2	2	1.45	1.45		
Blood.	400	530	550	54	917	1421	2448		
Cl.	1.0	1.4	1.0	0.9	1.2	1.2	0.9		
Sp. Gr.	1010	1007	1010	1013	1012	1012	1014		
Amount.	440	530	440	360	660	480	450		
Urobilin.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.		
Blood Volume.	1736								
Test Meal.									

Name. Patrick Campbell. Aged 7 years. Page 1.

Date.	30 Nov.	4 Dec.	15 Dec.	20 Dec.	28 Dec.	4 Jan.	11 Jan.	18 Jan	25 Jan.
Blood.									
R.B.C.(e)	4·035	3·940	4·065	4·365	4·220	4·265	3·985	3·620	3·640
R.B.C.(v)									
W.B.C.(e)	11,400	9,100	13,200	11,800	12,500	8,300	12,400	13,600	10,300
W.B.C.(v)									
Hb. (e)	40	46	80	83	82	80	76	62	64
Hb. (v)									
C.I.(e)	0·9	0·9	1·0	1·0	1·0	0·9	1·0	0·9	0·9
C.I.(v)									
Reticulocytes.	2·9	1·0	1·4	–	1·3	1·3	–	3·1	2·2
Film.	✓								
Blood Pressure.	134/80	128/40	110/68	104/46	98/60	104/54	102/62	96/40	102/50
Weight.	20·52	19·64	19·68	19·64	19·80	19·80	19·80	19·00	19·40
Oedema.	+	–	–	–	–	–	–	–	–
N.P.N.	41·0		42·0		43·9		38·2		48·5
Blood Cl.	426		546		463		495		514
Plasma Cl.	611		654		709		686		–
R.B.C. (Vol.)	36·4		38·4		39·3		38·2		33·3
Serum Protein.	5·49		5·43		5·79		5·29		6·04
Van den Berg.	Neg.		Neg.		Neg.		Neg.		Neg.
Urine.									
Alb.	1·0	1·0	1·5	1·0	0·5	3·5	1·0	0·5	1·0
Blood.	12	–	84	–	118	?	?	11	131
Cl.	9·4	4·8	8·2	5·0	2·0	1·4	2·1	2·8	3·8
Sp. Gr.	1013	1016	1015	1013	1018	1018	1014	1014	1020
Amount.	1240	410	1050	910	420	430	400	530	440
Urobilin.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Blood Volume.	1934								
Test Meal.									

Treatment: 15th Nov. Ferrous Sulphate 6 grains thrice daily.
28th Jan. Stop Ferrous Sulphate.

Name. Patrick Campbell. Aged 7 years. Page 2.

Date.	1 Feb.	4 Feb.	14 Feb.	21 Feb.	28 Feb.	4 Mar.	14 Mar.	20 Mar.	28 Mar.
Blood.									
R.B.C.(e)	3·940	3.640	3·800	3·540	3·945	3·915	3·905	4·965	4·055
R.B.C.(v)									
W.B.C.(e)	10,400	9,900	10,300	11,500	12,800	13,500	14,500	21,000	11,800
W.B.C.(v)									
Hb. (e)	40	68	46	64	42	44	44	82	46
Hb. (v)									
C.I.(e)	0·9	0·9	1.0	0·9	0·9	1·0	0·9	0·9	0·9
C.I.(v)									
Reticulocytes.	3·1	3·0	2·5	2·5	1·5	1·0	0·8	2·4	0·9
Film.									
Blood Pressure.	106/64	104/52	104/50	122/80	104/56	110/42	104/50	104/56	116/66
Weight.	19·80	19·92	19·24	19·80	20·0	19·92	20·24	20·0	20·0
Oedema.	–	–	–	–	–	–	–	–	–
N.P.N.		20·2		35·2		41·0			
Blood Cl.		528		500		–			
Plasma Cl.		698		662		–			
R.B.C. (Vol.)		34·5		33·9		34·3			
Serum Protein.		5·49		5·62		5·98			
Van den Berg.		0·5		Neg.		Neg.			
Urine.									
Alb.	1·0	1·45	1·5	2·0	1·0	1·0	1·0	1·5	2·5
Blood.	–	–							
Cl.	3·0	6·2	6·4	4·4	4·4	3·9	3·5	5·1	5·8
Sp. Gr.	1018	1016	1015	1013	1014	1018	1018	1020	1020
Amount.	600	820	860	980	460	400	580	960	610
Urobilin.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Blood Volume.									
Test Meal.		✓							

Treatment: See back.

28th Jan. Liver extract 2 c.cs. I.M. daily.
18th Feb. Stop Liver extract.

22nd Feb. Ascorbic acid 500 mgm. I.M. daily.
7th Mar. Stop I.M. ascorbic acid.
8th Mar. Ascorbic acid 500 mgm. by mouth daily.
23rd Mar. Stop oral ascorbic acid.

5th Apr. Ferrous Sulphate 3 grains thrice daily.

Name. Patrick Campbell. Aged 7 years.

Date.	4 Apr.	4 May	8 May						
Blood.									
R.B.C.(e)	4·050	4·015	4·345						
R.B.C.(v)									
W.B.C.(e)	11,000	9,900	10,200						
W.B.C.(v)									
Hb. (e)	46	44	45						
Hb. (v)									
C.I.(e)	0·9	0·9	0·9						
C.I.(v)									
Reticulocytes.	0·8								
Film.									
Blood Pressure.	124/68								
Weight.	20·80								
Oedema.	–	–	–						
N.P.N.	58·8								
Blood Cl.	436								
Plasma Cl.	–								
R.B.C. (Vol.)	38·8								
Serum Protein.	5·90								
Van den Berg.	Mes.								
Urine.									
Alb.	++	++	++						
Blood.									
Cl.									
Sp. Gr.									
Amount.									
Urobilin.									
Blood Volume.	2118								
Test Meal.									

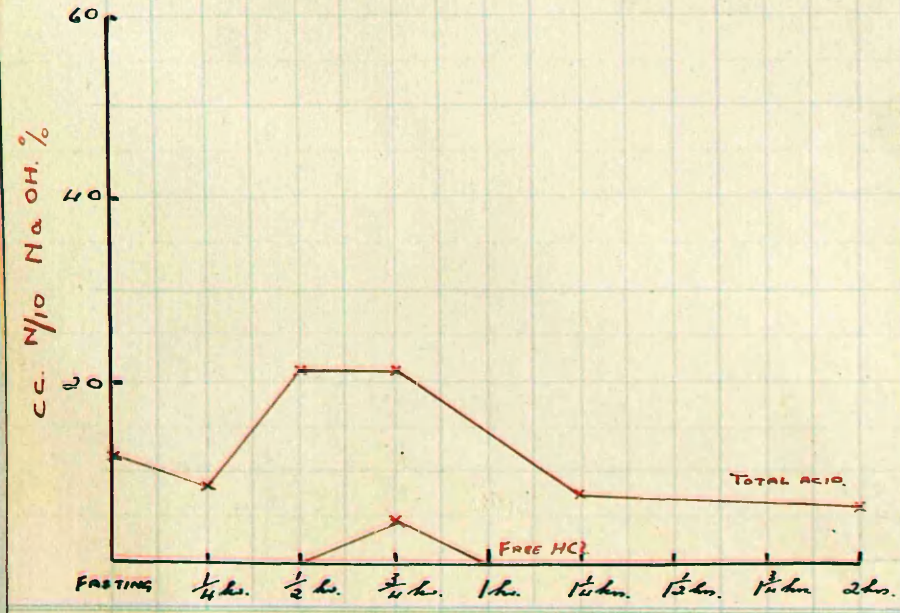
APPENDIX 2.

FRACTIONAL TEST MEALS IN PATIENTS WITH NEPHRITIS.

MADGE SWEENEY, 9yrs. W.a.h.

R.B.C. 3·940 m. per c.mm.

No oedema.

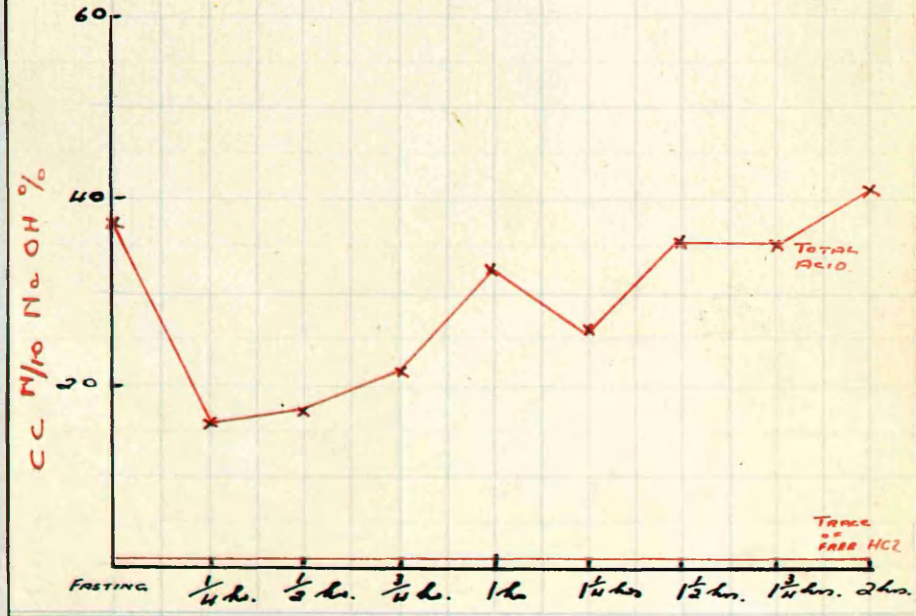


STARCH	+	++	++	+	−			−
MUCUS	∓	∓	∓	−	−			−

FULTON GILLIES, 10yrs. W.a.h.

R.B.C. 4·525 m. per c.mm.

No oedema.

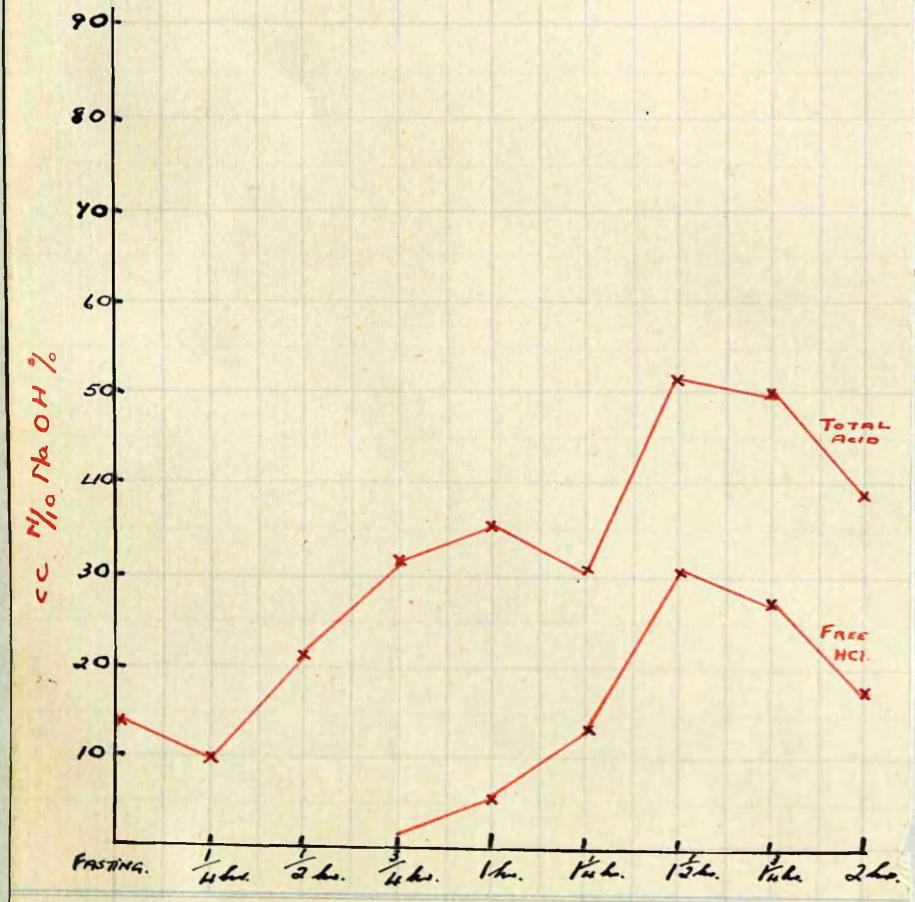


STARCH	+	+	+	+	+	+	+	+	
MUCUS	∓	−	−	∓	−	−	−	−	

Douglas Taylor, 11yrs. Wd. 4.

R.B.C. 4·400 m. per c.mm.

No oedema.

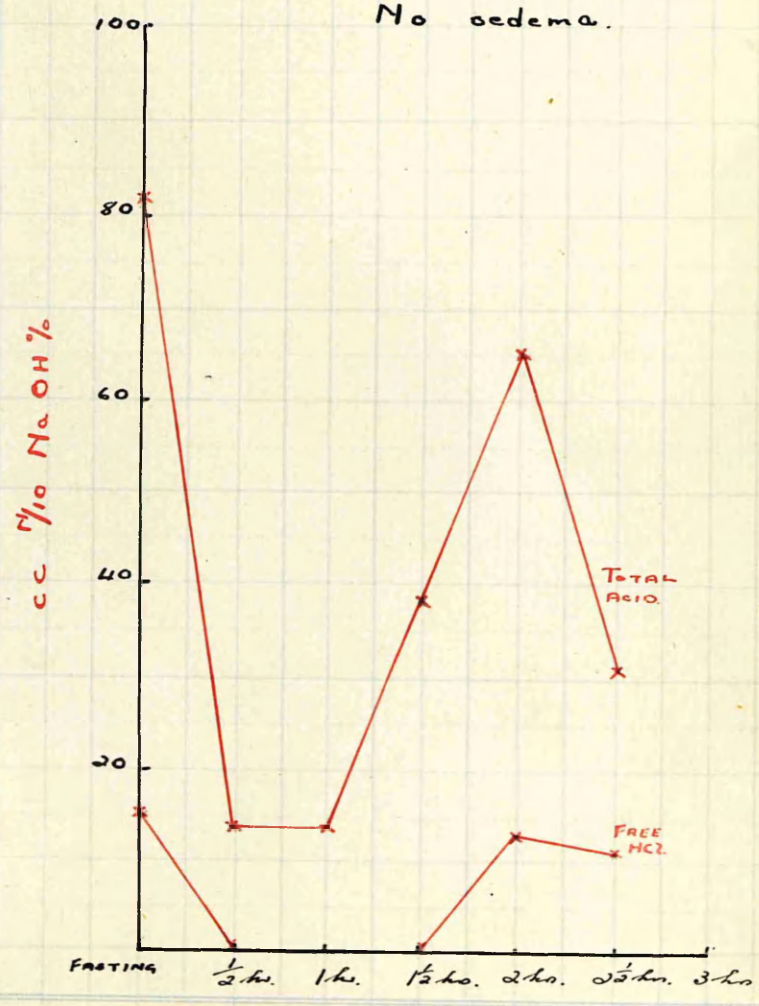


Starch	−	−	+	+	+	+	+	+	+
Mucus	++	++	++	++	++	+	+	+	+

Wm. Hendry, 4yrs. Wd. 5.

R.B.C. 3·980 m. per c.mm.

No oedema.

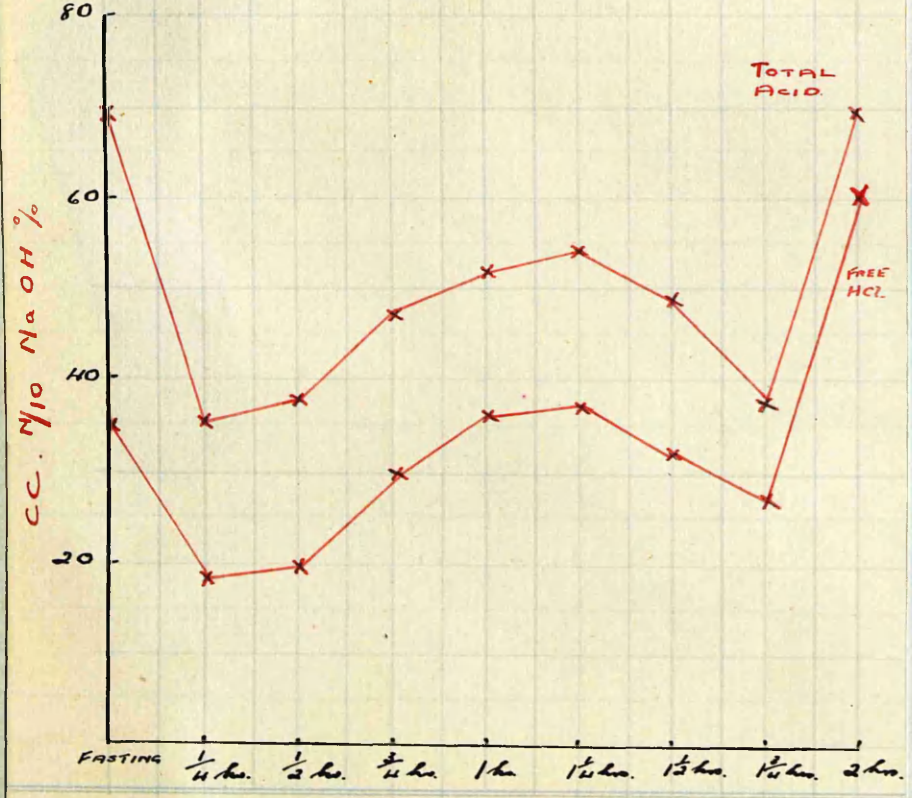


Starch	−	−	+	+	+	+
Mucus	∓	∓	∓	−	−	−

Mary McGhie Aet. 8yrs. Wd. 5.

R.B.C. 4.045 m. per c.mm.

No oedema.

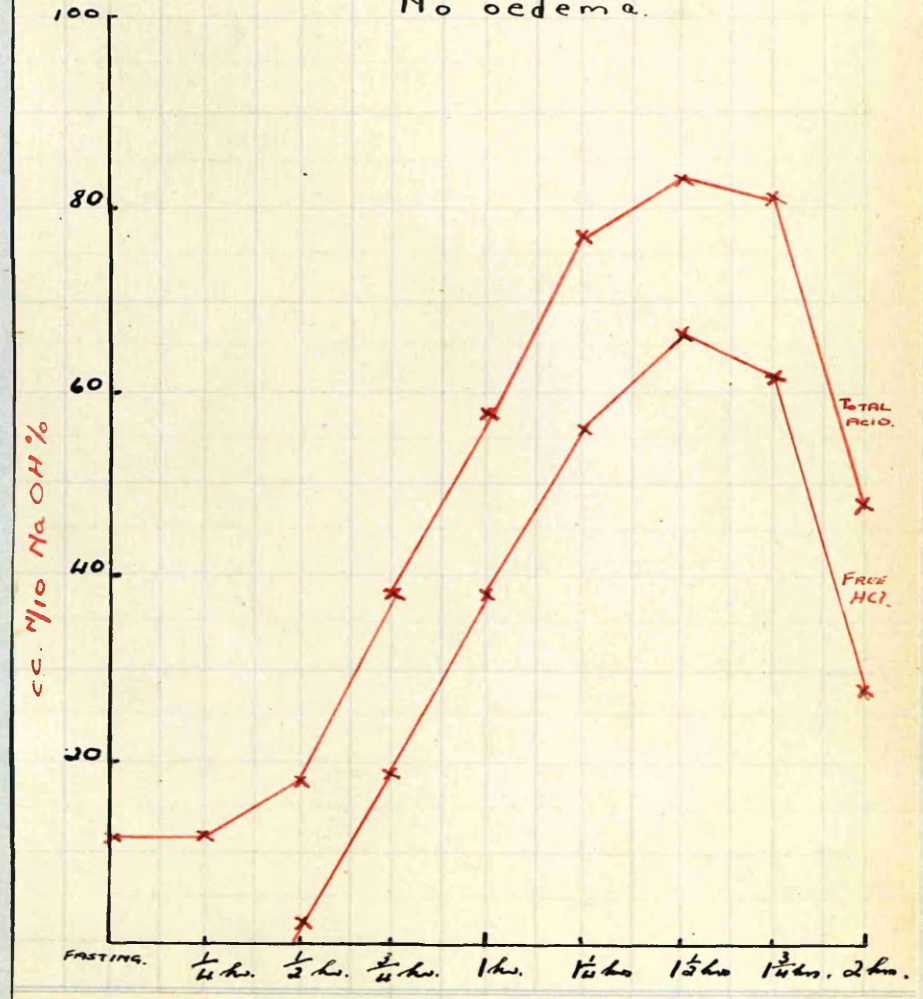


Starch	−	#	#	#	#	#	+	+	∓
Mucus	−	−	−	−	−	−	−	−	−

George Coyle 4yrs. Wd. 4.

R.B.C. 3.420 m. per c.mm.

No oedema.

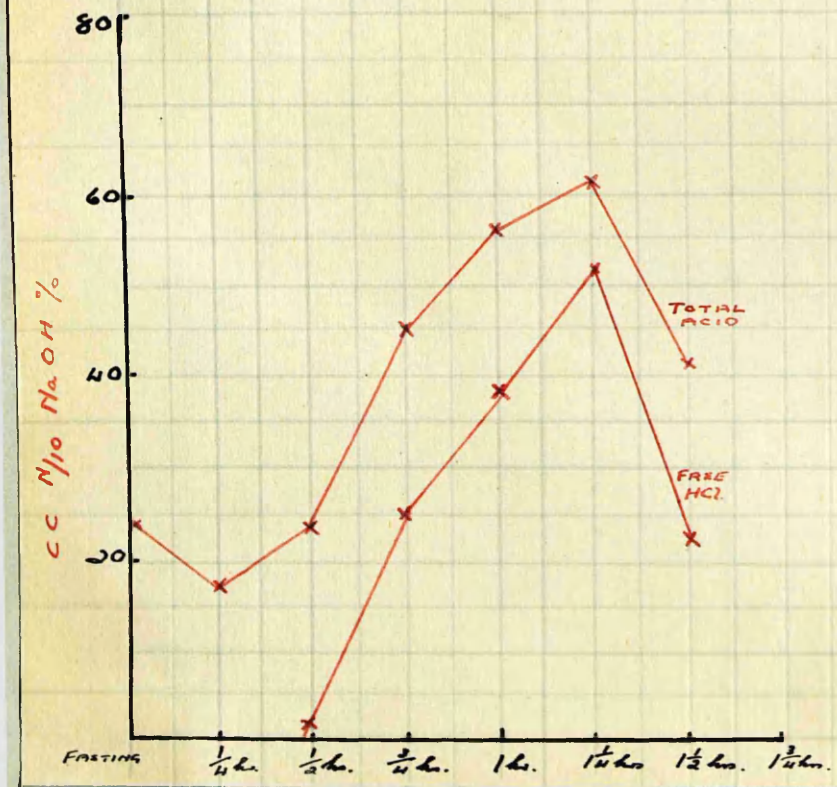


Starch	−	+	+	+	+	+	+	∓	∓
Mucus	−	−	−	−	−	−	−	−	−

GEORGE COYLE AET 4YRS. WO. 4.

R.B.C. 4·000 m. per c.mm.

No oedema.

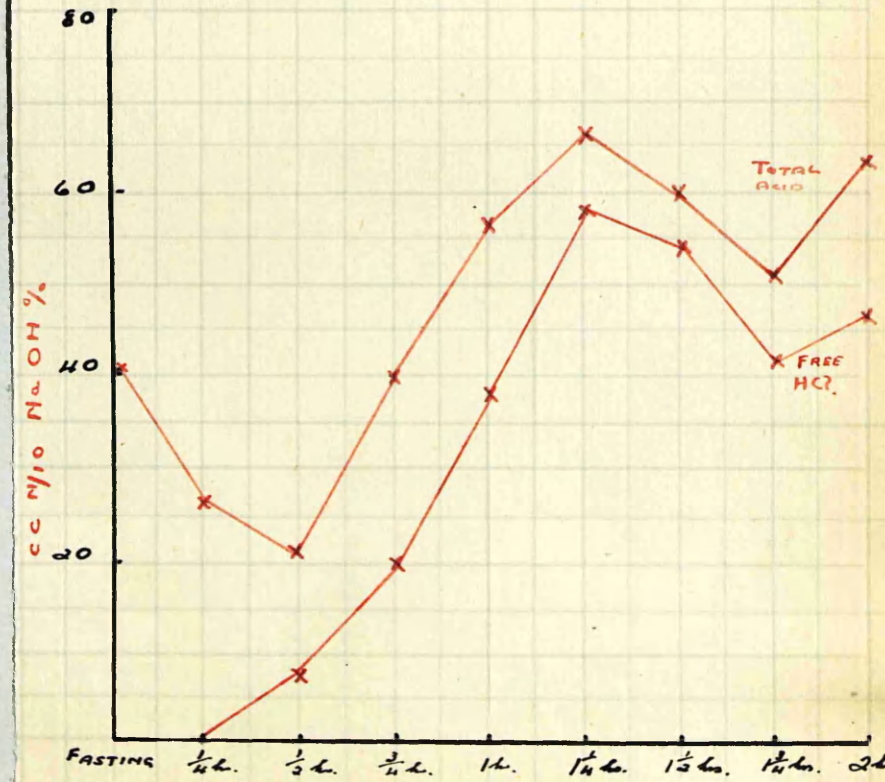


STARCH	—	++	++	++	++	++	+	∓
MUCUS	+	+	∓	∓	∓	∓	∓	—

ANNIE ANDERSON, AET. 8YRS. WO. 6.

R.B.C. 4·080 m. per c.mm.

Oedema +

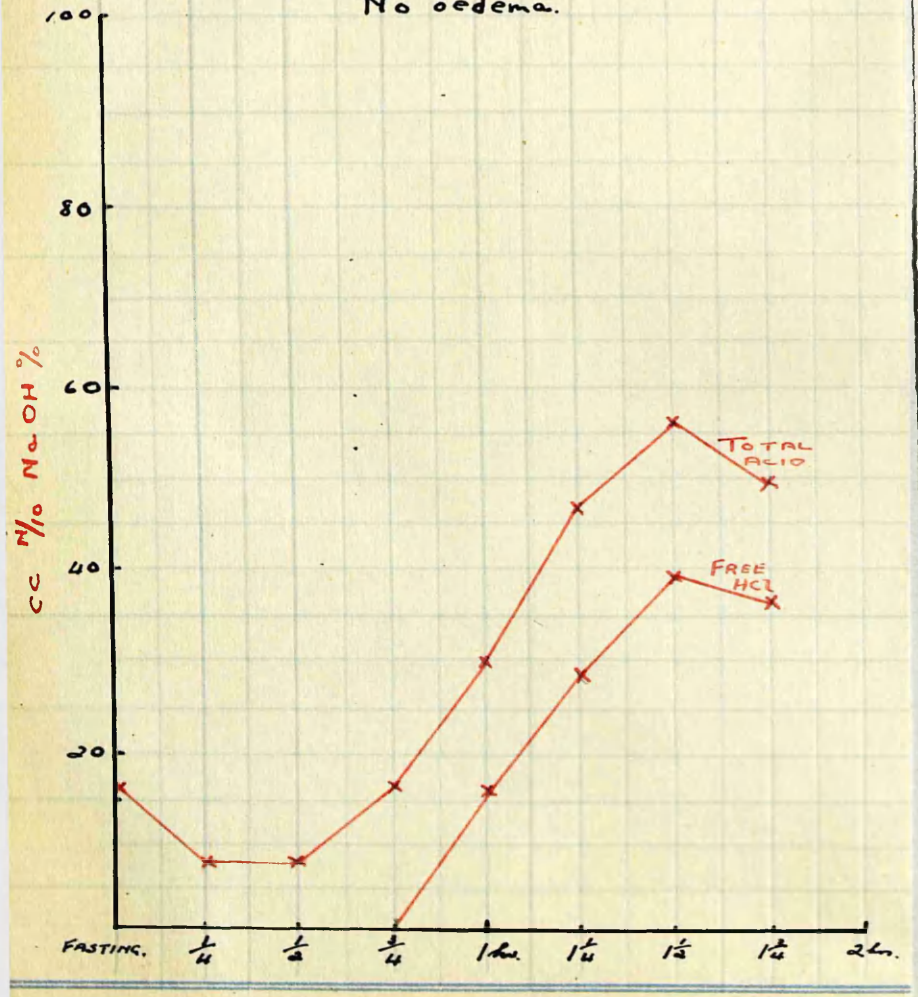


STARCH	+	+	+	+	+	—	—	—
MUCUS	+	+	+	∓	—	—	—	—	—

GEORGE HAMEY AET 8yrs. WOH.

R.B.C. 3·870 m. per c.mm.

No oedema.

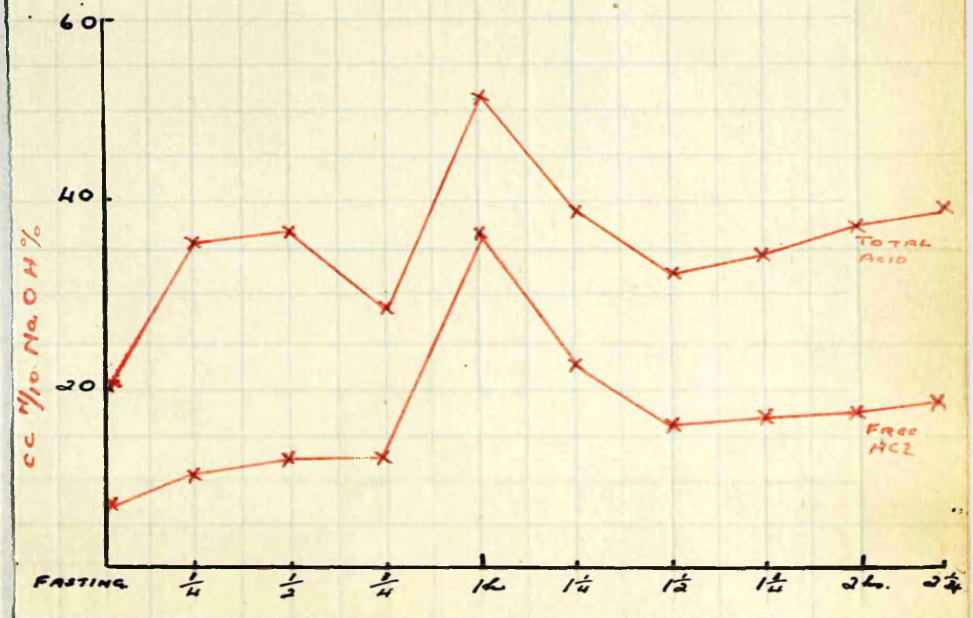


STARCH	−	+	+	+	+	+	+	+	+
MUCUS	+	∓	∓	−	−	−	−	−	−

JACK CAMPBELL AET 5yrs. WOS.

R.B.C. 2·430 m. per c.mm.

No oedema.

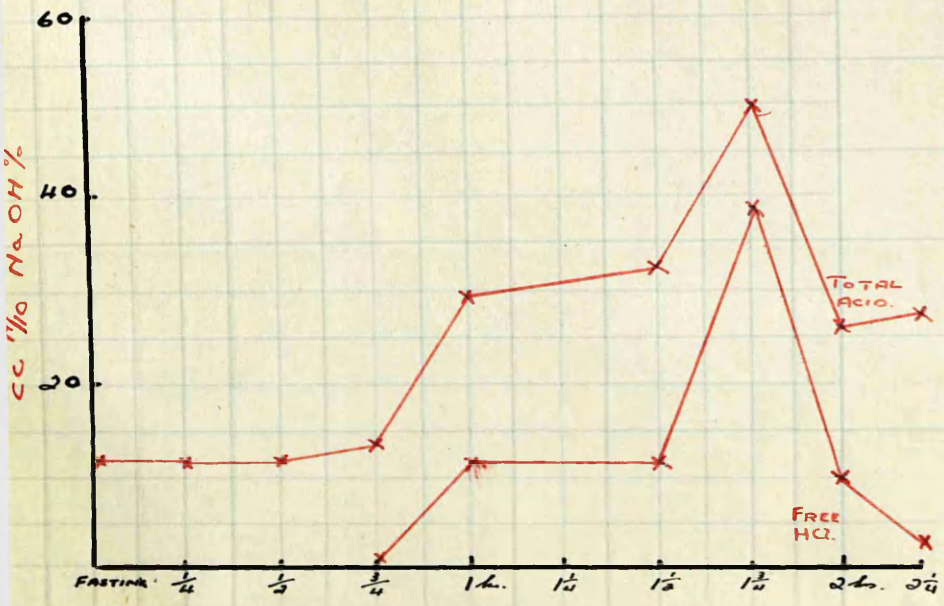


STARCH	−	+	+	+	+	+	∓	∓	−	−
MUCUS	+	∓	−	−	−	−	−	−	−	−

MARJORIE ROBERTSON AET 5YRS. WD. H.

R.B.C. 2.945 m. per c.mm.

Oedema ∓

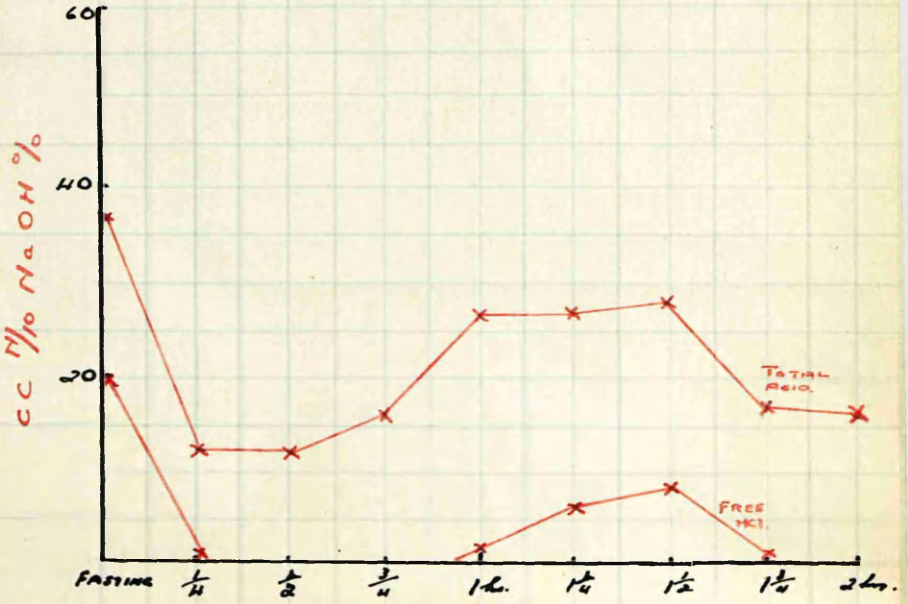


MUCUS	+	+	+	+		−	−	−	−
STARCH	−	+	+	+	+	+	+	+	+
BILE	−	−	−	−	−	−	−	+	+

ELIZABETH McINTOSH AET 5YRS. WD. H.

R. B. C. 5·220 m. per c.mm.

Oedema +

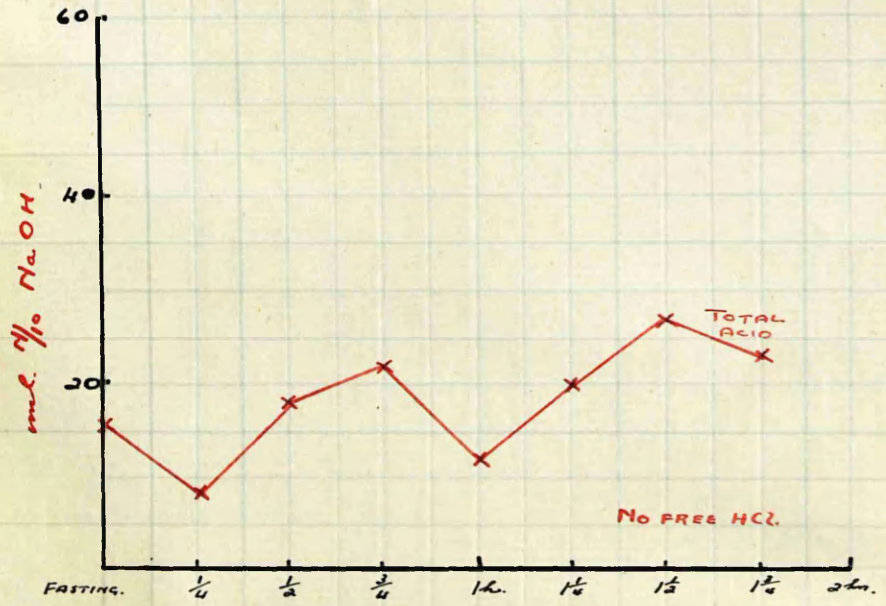


MUCUS	−	∓	∓	∓	∓	∓	∓	−	−
STARCH	−	+	+	+	+	+	+	∓	−
BILE	−	−	−	−	−	−	−	+	+

ANTONIO JACONELLI AET. 5YRS. WO.H.

R.B.C. 4·140 m. per c.mm.

No oedema.

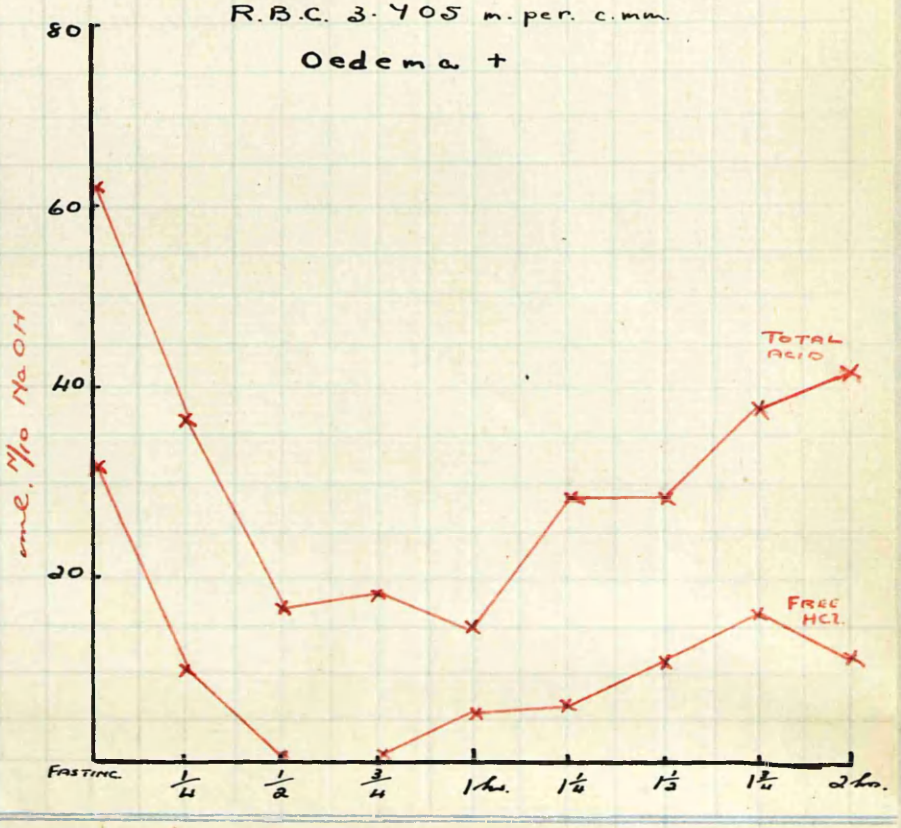


MUCUS	#	+	+	+	+	+	+	+
STARCH	—	—	—	—	—	—	—	—

ALEXANDRINA DAVIDSON AET. 10YRS. WO.H.

R.B.C. 3·705 m. per. c.mm.

Oedema +

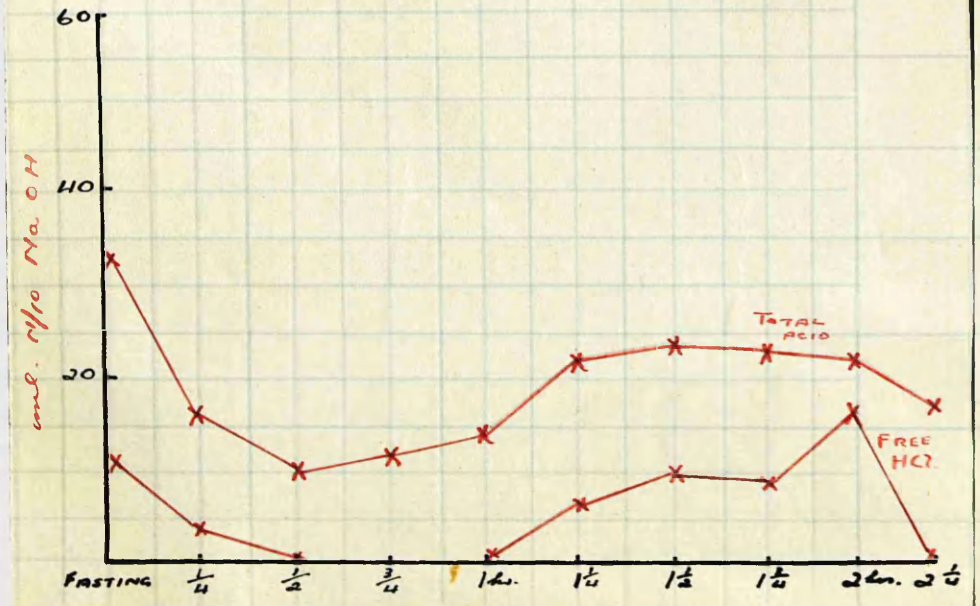


MUCUS	—	+	+	—	—	—	—	+	—
STARCH	—	+	+	+	+	+	+	+	+

PATRICK CAMPBELL AET. 4YRS. WDI

R.B.C. 3·640 m. per c.mm.

No oedema.

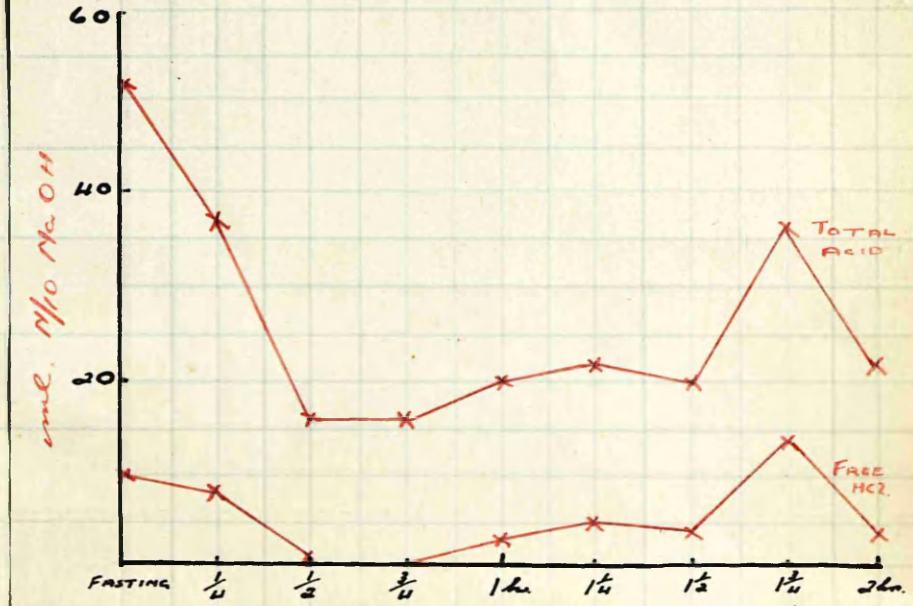


MUCUS	−	−	−	−	−	−	−	−	−	−
STARCH	−	−	−	−	−	+	+	+	+	−

AGNES McADAM AET. 12YRS WD. H.

R.B.C. 4·440 m. per c.mm.

No oedema.

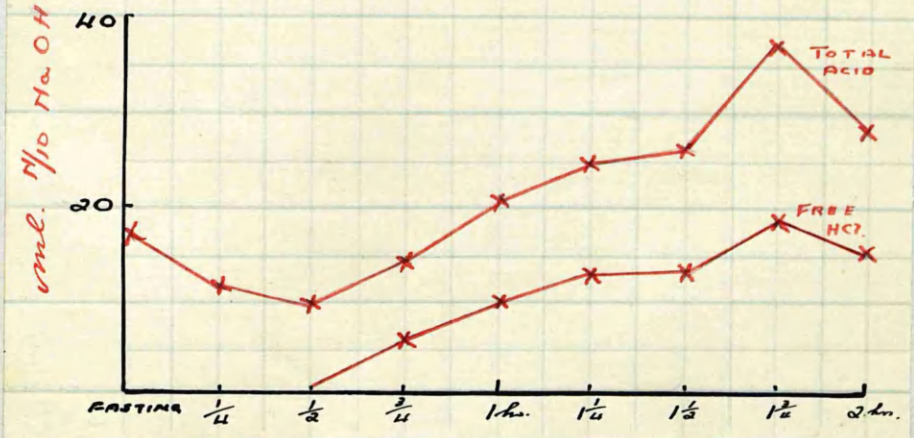


MUCUS	+	+	+	+	+	+	+	+	+
STARCH	−	+	+	+	+	+	+	+	+

JOHN REID AET. 4YRS. WD H.

R.B.C. 3,945,000 per c.mm.

No oedema.

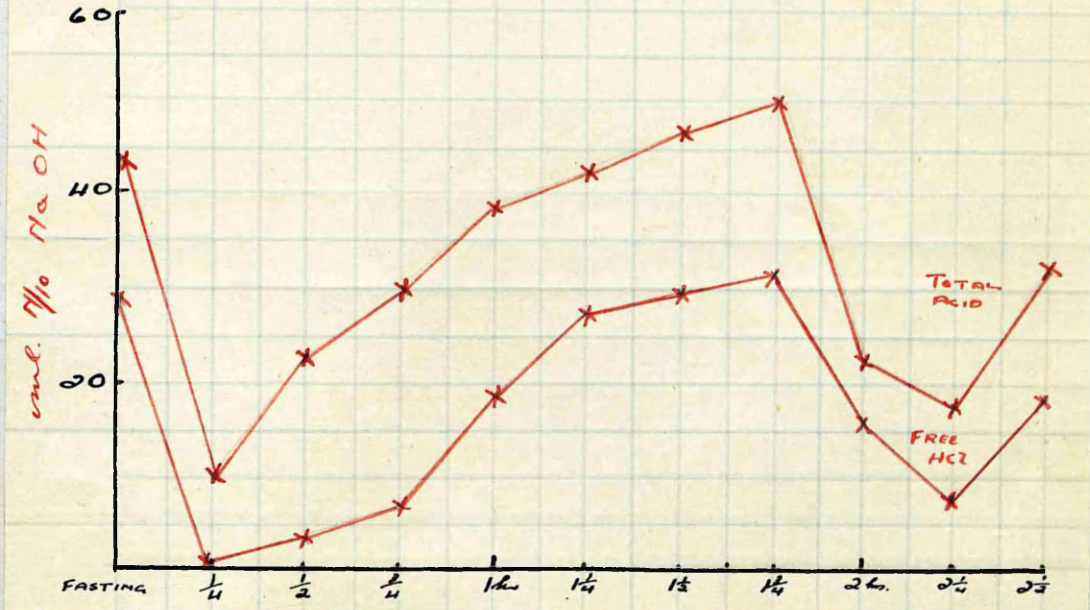


MUCUS	+	+	+	+	+	+	+	+	+
STARCH	−	+	+	+	+	+	+	+	+

EVELYN RAE AET. 5 YRS. W.O.I.

R. B. C. 4.440 m. per c. mm.

No oedema.



MUCUS	+	+	+	+	+	+	+	+	+	+
STARCH	+	+	+	+	+	+	+	∓	∓	−

APPENDIX THREE.

PROTOCOL OF BLOOD VOLUME ESTIMATION ON A CHILD.

Name.	George H.
Age.	8 yrs.
Weight.	23.40 Kilos.
Height.	121 cms.
Surface Area.	0.89 Sq.M.
Disease.	Ac. Nephritis. (Oedema ++) (Diuresis)

Haemoglobin: 80% (Haldane)
Red Cell Count: 4,130,000 per c.mm.
Blood Pressure: $110/50$ mm. Hg.

7 ml. of blood withdrawn from left elbow vein and added to 2 ml. 1.1% neutral potassium oxalate in 15 ml. graduated centrifuge tube, 4 ml. 1% congo red injected and needle withdrawn.

After exactly 4 mins. about 8 ml. blood withdrawn from right elbow vein and transferred to 2 ml. 1.1% oxalate in 15 ml. graduated centrifuge tube.

Thin layer of liquid paraffin added to each specimen and then centrifuged for 30 mins. at 3,000 revs./min. After centrifugalisation:-

Undyed Specimen.

Total volume in haematocrit	8.4 ml.
Red Cell Volume	2.65 ml.
Red Cell Volume %	41.4%
Dilution factor $\frac{\text{Plasma}}{\text{Oxalate + Plasma}}$	$\frac{375}{575}$

Dyed Specimen.

Total volume in haematocrit	9.3 ml.
Red Cell Volume	2.9 ml.
Red Cell Volume %	39.7%
Dilution Factor $\frac{\text{Plasma}}{\text{Oxalate + Plasma}}$	$\frac{44}{64}$

Contd.,

Standard Solution Congo Red $^{1}/_{400}$

Colorimeter Readings:

1.	9.15	
2.	9.0	9.0
3.	9.0	
4.	9.2	
5.	8.8	

$$\text{Plasma Volume} = \frac{4 \times 400 \times \frac{44}{64} \times 9.0}{10} + 4 = 996 \text{ ml.}$$

$$\therefore \text{ Blood Volume} = \frac{992 \times 100}{60.3} + 7 = 1650 \text{ ml.}$$

	Fraction of Body Weight.	ml./Kilo.	ml./Sq.m.	Total ml.
Red Blood Cells	-	27	730	654
Plasma	$^{1}/_{22.8}$	43	1119	996
Whole Blood	$^{1}/_{13.4}$	70	1849	1650

APPENDIX 4.

BLOOD VOLUME ESTIMATIONS IN NEPHRITIS.

Explanation of table.

1. Name.

2. Age in years.

3. Date - day and month.

4. Weight in kilograms on day of estimation.

5. Oedema-free weight i.e. the lowest weight which the patient reached subsequently when all oedema had disappeared.

6. Height in centimetres on day of estimation.

7. Surface Area in Square Metres calculated from the monogram of Dubois (Dubois 1927).

8. Blood Pressure in millimetres of mercury on the day of the estimation.

9. Oedema indicated by empirical signs (Rennie, 1933)

   ++ = General anasarca with effusions into serous cavities.

   + = Well marked oedema with pitting on pressure.

   $\mp$ = Ruffiness of the face and a subsequent fall in weight with diuresis.

   - = No oedema.

10. Haemoglobin of the blood in per cent Haldane.

11. Red cell count of capillary blood in millions per cubic millimetre.

12./

BLOOD VOLUME ESTIMATIONS IN NEPHRITIS.

1 NAME	2 AGE yrs.	3 DATE	4 WEIGHT Kg.	5 Wt. (o.f.) Kg.	6 HEIGHT Cms.	7 S.A. Sq.M.	8 B. P. mm.Hg.	9 Oedema	10 Hb. %H.	11 R. B. C. mils/c.mm.	12 W. B. C. per c.mm.	13 HAEM. %	14 S.P. g.%	15 RED CELL VOLUME (a) ml./Kg.	(b) ml./Kg.(o.f.)	(c) ml./Sq.M.	(d) TOTAL ml.	16 PLASMA VOLUME (a) Fract. B.Wt.	(b) Fract. B.Wt.(o.f.)	(c) ml./Kg.	(d) ml./Kg.(o.f.)	(e) ml./Sq.M.	(f) TOTAL ml.	17 BLOOD VOLUME (a) Fract. B.Wt.	(b) Fract. B.Wt.(o.f.)	(c) ml./Kg.	(d) ml./Kg.(o.f.)	(e) ml./Sq.M.	(f) TOTAL ml.	18 D.
George Coyle	7	18 Mch.	21·86	17·6	112	0·82	126/86	+	72	3·660	14,800	34·6	6·94	21	26	562	461	1/24·5	1/19·8	40	49	1055	865	1/15·5	1/12·9	61	75	1617	1326	A.N
" "	7	20 June	20·72	"	112	0·79	118/60	−	82	4·125	15,900	44·0	7·82	34	40	899	710	1/22·3	1/19	44	51	1141	901	1/12·1	1/10·6	78	91	2039	1611	"
Frank Conway	6	21 April	19·56	15·2	104	0·73	172/106	+	74	4·265	14,200	38·7	5·87	32	41	853	622	1/19·2	1/15·8	50	65	1350	986	1/11·5	1/9·4	82	106	2203	1608	"
" "	6	26 May	16·40	"	104	0·68	120/80	−	90	4·770	7,900	43·2	7·76	30	32	718	488	1/24·7	1/22·9	39	43	949	645	1/13·7	1/12·6	69	75	1666	1133	"
Robert McPhee	4	12 Aug.	13·64	13·6	99	0·60	102/78	−	70	3·985	12,500	36·3	8·24	30	30	682	409	1/18·3	1/18·3	53	53	1195	717	1/11·4	1/11·4	83	83	1877	1126	"
Antonio Jaconelli	5	12 Dec.	19·11	16·3	113	0·77	150/100	+	72	4·025	13,200	36·1	6·32	33	38	809	623	1/17·1	1/14·6	56	67	1407	1083	1/10·6	1/9	89	105	2216	1706	"
" "	5	24 Jan.	16·81	"	113	0·73	102/50	−	64	3·740	14,100	35·4	7·74	30	31	696	508	1/17·6	1/17·1	55	57	1267	925	1/11·1	1/10·7	85	88	1963	1433	"
Eleanor Davidson	10	24 Jan.	29·61	23·6	134	1·05	138/70	+	72	3·705	13,000	39·0	6·57	31	39	872	916	1/20·1	1/16	48	61	1365	1433	1/11·9	1/9·5	79	100	2237	2349	"
" "	10	31 Jan.	25·08	"	133	0·97	106/46	−	80	4·175	14,400	40·3	8·56	28	30	724	702	1/23·4	1/22·1	41	44	1071	1039	1/13·6	1/12·8	69	74	1795	1741	"
" "	10	6 Mch.	25·21	"	134	0·98	96/62	−	84	4·630	15,500	41·8	8·13	46	49	1182	1158	1/15·2	1/14·2	64	68	1646	1613	1/8·6	1/8	110	117	2828	2771	"
Robt. Jamieson	10	10 Mch.	25·58	25·58	133	0·98	124/74	−	70	3·685	11,700	36·1	7·63	32	32	849	832	1/16·9	1/16·9	58	58	1504	1474	1/10·5	1/10·5	90	90	2353	2306	4
Edward Gorman	8	10 April	23·24	18·9	118	0·87	110/80	+	81	4·130	12,400	41·7	6·55	33	40	878	764	1/21·1	1/17·1	46	57	1226	1067	1/12.	1/9·7	79	97	2104	1831	"
" "	8	17 April	19·88	"	118	0·81	90/60	−	89	4·610	14,700	44·8	7·89	40	42	972	787	1/19·9	1/18·9	49	51	1200	972	1/10·7	1/10·1	89	93	2172	1759	"
" "	8	9 May	20·96	"	119	0·83	100/70	−	72	3·875	8,700	38·3	7·67	41	46	1034	858	1/14·7	1/13·2	66	73	1668	1385	1/8·8	1/7·9	107	118	2702	2243	"
Robert Davis	10	30 April	27·0	23·0	131	0·99	158/110	+	66	3·570	15,600	32·6	7·11	28	33	769	761	1/16·7	1/14·2	58	68	1589	1573	1/10·9	1/9·4	86	101	2358	2334	"
" "	10	6 May	23·0	"	131	0·93	106/58	−	72	4·115	15,000	40·0	8·57	34	34	853	793	1/18·8	1/18·8	52	52	1279	1190	1/10·1	1/10·1	86	86	2132	1983	"
Evelyn Gillespie	10	2 may	23·48	19·4	121	0·89	150/90	+	74	3·685	14,100	37·5	6·65	29	36	775	690	1/19·8	1/16·4	49	59	1293	1151	1/12	1/9·9	78	95	2068	1841	"
" "	10	9 May	20·6	"	119	0·83	112/68	−	83	4·780	19,600	42·9	8·91	36	38	884	734	1/20·5	1/19·3	47	50	1178	978	1/11·3	1/10·7	83	88	2062	1712	"
James McKenna	5	26 July	20·52	14·4	108	0·77	104/?	+ +	72	4·185	11,000	40·5	5·44	29	44	773	595	1/22·6	1/15·9	43	61	1145	882	1/13·1	1/9·2	72	133	1918	1477	"
" "	5	2 Aug.	16·12	"	107	0·71	90/74	+	76	4·580	8,400	45·3	6·04	40	47	904	642	1/20·5	1/18·3	47	53	1075	763	1/10·8	1/9·7	87	98	1979	1405	"
" "	5	9 Aug.	15·44	"	107	0·68	78/60	−	94	4·960	10,300	45·6	6·22	60	68	1351	919	1/13·8	1/12·8	70	76	1600	1088	1/7·3	1/6·8	130	139	2952	2007	"
Sarah Doris	4	30 July	17·16	14·74	102	0·68	126/48	+	56	3·655	8,300	35·2	7·10	29	34	731	497	1/19·2	1/16·5	51	59	1278	869	1/11·9	1/10·1	80	93	2009	1366	"
" "	4	7 Aug.	15·0	"	101	0·64	104/?	−	72	4·140	11,100	42·0	8·49	34	34	787	504	1/22·6	1/22·2	43	44	1006	644	1/12·3	1/12·1	77	78	1794	1148	"
George Hainey	8	29 May	27·16	19·6	121	0·95	106/?	+ +	92	4·520	11,900	43·9	5·64	28	40	823	782	1/25·7	1/18·6	38	52	1078	1024	1/13·4	1/10·2	66	92	1901	1806	"
" "	8	13 June	23·4	"	121	0·89	110/?	+	80	4·130	13,100	39·7	6·37	27	33	730	654	1/22·8	1/19·1	43	51	1119	996	1/13·4	1/11·2	70	84	1849	1650	"
James McDowall	5	2 Feb.	24·4	18·6	107	0·83	98/56	+ +	106	5·135	14,000	49·3	—	46	61	1358	1127	1/20	1/15·6	47	62	1395	1158	1/10·1	1/7·7	94	123	2753	2285	N.V.
" "	5	6 Mch.	18·84	"	107	0·74	96/32	−	98	4·730	9,400	46·4	—	65	65	1641	1214	1/12	1/12	74	75	1895	1402	1/7·2	1/7·2	139	140	3536	2616	"
" "	5	20 April	18·84	"	107	0·76	106/58	−	98	4·880	12,200	42·7	6·12	46	46	1133	861	1/15·8	1/15·6	61	62	1518	1154	1/8·8	1/8·7	107	108	2651	2015	"
" "	6	11 Nov.	22·88	"	112	0·83	106/76	+ +	110	5·470	12,900	50·0	5·98	51	63	1416	1175	1/18·9	1/15·4	51	63	1416	1175	1/9·2	1/7·5	102	126	2832	2350	"
" "	6	28 Nov.	18·66	"	111	0·75	106/60	−	88	4·505	9,200	43·7	6·14	52	52	1280	960	1/14·7	1/14·6	66	66	1648	1236	1/8·0	1/8·0	118	118	2928	2196	"

BLOOD VOLUME ESTIMATIONS IN NEPHRITIS (CONTINUED).

NAME	AGE yrs.	DATE	WEIGHT Kg.	Wt.(O.F) Kg.	HEIGHT Cms.	S. A. Sq.M.	B. P. mm. Hg.	Oedema	Hb. % H.	R. B. C. mils/c.mm.	W. B. C. per c.mm.	HAEM. %	S. P. g. %	RED CELL VOLUME (a) ml./Kg.	(b) ml./Kg.(O.F)	(c) ml./Sq.M.	(d) TOTAL ml.	PLASMA VOLUME (a) Fract. B. Wt.	(b) Fract. B.Wt.(O.F)	(c) ml./Kg.	(d) ml./Kg.(O.F)	(e) ml./Sq.M.	(f) TOTAL ml.	BLOOD VOLUME (a) Fract. B. Wt.	(b) Fract. B.Wt.(O.F)	(c) ml./Kg.	(d) ml./Kg.(O.F)	(e) ml./Sq.M.	(f) TOTAL ml.	D.
Thomas Moore	7	21 July	27·24	17·8	114	0·90	122/92	+ +	103	5·640	6,400	54·5	5·13	38	59	1159	1043	1/30·3	1/19·8	32	49	970	873	1/13·4	1/8·8	70	108	2129	1916	N.N.
" "	7	22 Aug.	24·36	"	114	0·80	130/90	+ +	60	3·280	20,000	34·0	—	36	41	911	729	1/14	1/12·2	69	80	1770	1416	1/9	1/7·8	105	121	2681	2145	"
" "	7	14 Nov.	22·8	"	117	0·85	108/68	—	96	5·225	8,600	45·5	7·32	35	45	942	801	1/32·4	1/17·5	43	55	1159	985	1/12	1/9·4	78	100	2101	1786	"
" "	7	16 Dec.	26·6	"	118	0·91	104/78	+	98	5·395	9,500	50·0	5·77	31·5	47	924	841	1/30·7	1/20·6	31·5	47	924	841	1/14·9	1/10	63	94	1848	1682	"
" "	7	10 Mch.	33·0	"	119	1·0	124/74	+ +	58	3·295	13,000	32·2	—	13	24	422	422	1/36	1/19·4	27	50	889	889	1/23·7	1/12·8	40	74	1311	1311	"
Isobel Dunn	5	25 Feb.	19·88	12·0	95	0·70	120/92	+ +	74	3·865	15,900	36·1	—	15	24	411	288	1/38·3	1/23·1	25	42	723	506	1/23·6	1/14·3	40	66	1134	794	"
William Glennie	7	21 Mch.	21·84	18·6	110	0·80	102/72	+ +	95	4·815	18,600	45·8	5·47	66	77	1792	1434	1/12·6	1/10·7	77	90	2103	1682	1/6·6	1/5·6	143	167	3895	3116	"
" "	7	6 June	18·64	"	111	0·76	88/68	—	92	4·915	18,100	47·5	—	46	46	1147	860	1/19·1	1/19·1	51	51	1267	950	1/9·5	1/9·5	97	97	2414	1810	"
Chas. McGoldrick	3	6 May	15·84	14·0	99	0·65	110/60	+	86	5·045	10,600	48·3	5·59	43	49	1046	680	1/21·1	1/18·7	46	52	1120	728	1/10·6	1/9·4	89	101	2166	1408	"
" "	3	2 June	18·04	"	99	0·68	98/58	+ +	86	4·985	16,900	46·3	5·15	37	48	982	668	1/22·6	1/17·5	43	55	1140	775	1/11·8	1/9·2	80	103	2122	1443	"
" "	3	14 July	14·21	"	98	0·62	124/100	—	75	4·135	12,600	42·9	—	39	40	898	557	1/18·6	1/18·3	52	53	1195	741	1/10·3	1/10·2	91	93	2093	1298	"
" "	3	9 Aug.	15·44	"	97	0·63	102/60	—	102	5·000	10,000	48·3	6·25	46	51	1124	708	1/20·5	1/18·6	47	52	1162	732	1/10·1	1/9·2	93	103	2286	1440	"
Jean Cook	6	14 July	12·72	12·72	97	0·62	192/150	—	86	4·375	11,700	43·7	6·85	46	46	929	576	1/16·6	1/16·6	58	58	1197	742	1/9·1	1/9·1	104	104	2126	1318	N.S.
Margt. McRiven	9	29 Aug.	16·68	16·68	123	0·77	194/110	—	108	5·415	7,800	44·7	—	49	49	1051	809	1/16·2	1/16·2	60	60	1300	1001	1/8·7	1/8·7	109	109	2351	1810	"
John Dryden	9	18 Nov.	16·0	16·0	98	0·65	88/60	—	74	4·260	10,800	38·8	7·35	39	39	962	625	1/15·7	1/15·7	62	62	1517	986	1/9·4	1/9·4	101	101	2478	1611	"
Robert Lowther	10	25 Jan.	17·84	17·84	113	0·75	90/?	—	43	2·205	6,900	21·8	10·41	16	16	367	275	1/17·5	1/17·5	55	55	1317	988	1/13·3	1/13·3	71	71	1684	1263	"
Patrick Breslin	7	5 June	14·0	14·0	99	0·61	110/70	—	58	3·890	10,000	34·4	9·23	27	27	623	380	1/18·8	1/18·8	52	52	1187	724	1/12	1/12	79	79	1810	1104	"
Annie Anderson	8	27 May	25·45	22·5	135	0·99	108/66	+	64	3·360	8,300	31·8	4·94	19	21	480	475	1/24·2	1/21·4	40	45	1029	1019	1/16·1	1/14·2	59	66	1509	1494	C.H.M.
Jack Campbell	5	19 July	17·4	16·5	107	0·71	100/70	∓	44	2·440	6,400	25·0	5·09	25	26	611	434	1/13	1/12·3	75	79	1834	1302	1/9·5	1/9	100	105	2445	1736	"
John Reid	7	12 June	35·68	28·08	142	1·20	124/78	+ +	72	3·725	6,000	39·2	7·2	33	41	965	1158	1/19	1/15	51	65	1519	1823	1/11·3	1/8·9	84	106	2484	2981	"
" "	7	20 June	30·12	"	141	1·10	110/66	+	80	4·565	5,900	41·6	6·81	40	42	1077	1185	1/17·6	1/16·4	55	59	1511	1662	1/10	1/9·3	95	101	2588	2847	"
" "	7	4 July	30·32	"	141	1·13	98/66	+	75	4·090	6,100	40·8	6·65	40	43	1064	1202	1/16·1	1/14·9	60	65	1620	1831	1/9·4	1/8·7	100	108	2684	3033	"
Patrick Campbell	7	30 Nov.	20·52	18·0	118	0·82	134/80	+	70	4·035	11,400	36·4	5·49	34	39	859	704	1/16·2	1/14·2	60	68	1500	1230	1/10	1/8·8	94	107	2359	1934	"
" "	7	4 April	20·8	"	116	0·82	124/68	+	76	4·050	11,000	37·0	5·90	38	44	956	784	1/15·1	1/13·1	64	74	1627	1334	1/9·2	1/8	102	118	2583	2118	"
Marjorie Robertson	6	9 Jan.	14·8	13·8	106	0·66	130/70	—	58	2·895	7,600	28·7	10·73	24	26	542	358	1/16·2	1/15·1	60	64	1344	887	1/11·2	1/10·5	84	90	1886	1245	"
	6	9 Feb.	15·2	"	106	0·67	102/60	—	60	2·845	8,300	30·3	—	28	30	625	419	1/15·3	1/13·9	63	70	1439	964	1/10·4	1/9·4	91	100	2064	1383	"
	6	3 April	17·2	"	106	0·70	120/70	—	62	3·485	11,800	35·2	8·13	28	35	690	483	1/18·8	1/15·1	52	64	1270	889	1/11·8	1/9·5	80	99	1960	1372	"
Agnes McAdam	12	13 Mch.	29·68	28·4	144	1·11	124/64	—	85	4·845	8,400	42·4	7·94	34	36	911	1011	1/21	1/20·1	46	48	1237	1373	1/11·7	1/11·2	80	84	2148	2384	"

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